

National Institute
of
Allergy and Infectious
Diseases

Annual Report of Program Activities

October 1, 1982 - September 30, 1983

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

Gifts, 6/3/83

National Institute
of
Allergy and Infectious
Diseases (U.S.)

Annual Report of **Program Activities**

October 1, 1982 - September 30, 1983

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

For Administrative Use

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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1983 ANNUAL REPORT PROJECT NUMBER LISTING*

<u>Z01 AI</u>	<u>Z01 AI</u>
00006-12 LMM	00097-25 LPD
00011-18 LVD	00098-27 LPD
00013-20 LMM	00099-13 LPD
00018-17 LMM	00102-09 LPD
00020-08 LVD	00103-16 LPD
00027-16 LMM	00108-12 LPD
00030-15 LI	00123-17 LBV
00035-08 LI	00126-10 LBV
00036-18 LI	00131-16 LMI
00037-16 LI	00134-21 LMI
00040-09 LI	00135-09 LVD
00043-18 LGI	00136-11 LMI
00045-15 LCI	00138-09 LVD
00047-14 LCI	00141-09 LMI
00048-13 LCI	00143-14 LMI
00057-10 LCI	00144-19 LMI
00058-09 LCI	00145-16 LMI
00061-21 EB	00146-10 LMI
00063-13 EB	00147-08 LI
00065-10 LMSF	00148-08 LI
00069-22 LMSF	00154-08 LCI
00071-12 OSD	00155-08 LCI
00072-12 LPVD	00161-06 LPD
00073-18 LPVD	00162-07 LPD
00074-11 LPVD	00166-06 LIG
00082-22 EB	00168-06 LIG
00085-06 LPVD	00169-06 LIG
00086-06 LPVD	00170-06 LIG
00094-24 LPD	00171-06 LIG
	00172-06 LIG

*Does not include terminated or inactive projects

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1983 ANNUAL REPORT PROJECT NUMBER LISTING

<u>Z01 AI</u>	<u>Z01 AI</u>
00173-06 LIG	00226-02 LI
00180-06 LIG	00227-02 LI
00182-05 LMSF	00228-02 LI
00183-05 LMSF	00229-02 LI
00186-10 LMI	00230-02 LMSF
00189-04 LCI	00231-02 LMSF
00190-05 LMM	00232-02 LMSF
00191-05 LIG	00233-02 LMSF
00192-05 LCI	00234-02 LMSF
00193-04 LMSF	00235-02 LMSF
00194-04 LMSF	00236-02 LMSF
00196-04 LMSF	00238-02 LMSF
00197-04 LPD	00239-02 LMSF
00199-04 LPVD	00240-02 LPD
00201-04 RMOB	00241-02 LPD
00203-04 LMI	00242-02 LPD
00205-03 LVD	00244-02 LPD
00208-03 LPD	00248-02 LPD
00210-03 LIR	00249-02 LCI
00211-03 LIR	00250-02 LCI
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00213-03 LIR	00253-02 LPD
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00218-02 LMM	00255-02 LPD
00219-02 LMM	00256-02 LPD
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00222-02 LMM	00258-02 LPD
00223-02 LI	00259-02 LI
00224-02 LI	00260-02 LPVD
00225-02 LI	00261-02 LPVD

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1983 ANNUAL REPORT PROJECT NUMBER LISTING

Z01 AI

00262-02 LPVD
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00264-02 LPVD
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00266-02 LPVD
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00296-02 LBV

Z01 AI

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NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

1983 ANNUAL REPORT PROJECT NUMBER LISTING

<u>Z01 AI</u>	<u>Z01 AI</u>
00328-02 LID	00361-01 LCI
00329-02 LID	00362-01 LMSF
00330-02 LID	00363-01 LMSF
00331-02 LID	00364-01 LMSF
00332-02 LID	00365-01 LID
00333-02 LID	00366-01 LID
00334-02 LID	00367-01 LID
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00340-02 LID	00371-01 LID
00341-02 LID	00372-01 LID
00342-02 LID	00373-01 LID
00343-02 LID	00374-01 LID
00344-02 LID	00375-01 LVD
00345-02 LID	00376-01 LVD
00346-02 LID	00377-01 LVD
00347-01 LPD	00378-01 OSD
00348-01 LPD	00379-01 LCI
00349-01 LI	00380-01 LPVD
00350-01 LPD	00381-01 LIR
00351-01 LPD	00382-01 LIR
00352-01 LIG	00383-01 OSD
00353-01 LMM	00384-01 OSD
00354-01 LCI	00385-01 OSD
00355-01 LCI	
00356-01 LCI	
00357-01 LCI	
00358-01 LCI	
00359-01 LCI	
00360-01 LCI	

ANNUAL REPORT OF PROGRAM ACTIVITIES
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
October 1, 1982 through September 30, 1983

Fiscal Year 1983 can be characterized as one of increasing scientific opportunities for the NIAID community. Some of the scientific opportunities are being attributed to "the biologic revolution," based upon recombinant DNA technology, hybridoma technology and an increasing understanding of the immune system.

Dr. Richard M. Krause, Director of the National Institute of Allergy and Infectious Diseases (NIAID) continues to be a forceful spokesman for the Institute, whether testifying before Congressional committees, addressing national or international scientific groups, or discussing NIAID's research programs with professional societies or volunteer health organizations.

During this fiscal year, NIAID expended a great amount of effort on Acquired Immune Deficiency Syndrome (AIDS)--in addition to its other major research activities on allergy, immunology and infectious diseases. AIDS, which destroys the body's immune system, has been labeled the "Nation's number one health priority" by the Public Health Service. NIAID's Laboratory of Immunoregulation, headed by Dr. Anthony Fauci, is currently treating AIDS patients in exciting research on this disease. Many other NIAID intramural laboratories are actively involved in a search for the putative agent which causes AIDS. Extramurally, there has been a huge increase in NIAID funding for grants and contracts to study AIDS.

In May, the Director headed a PHS team which visited Haiti to exchange information on the occurrence and the clinical nature of AIDS in Haiti and the United States; discussions were held on possible opportunities to extend research cooperation between medical scientists of the two countries concerning the etiology, epidemiology, pathogenesis, and treatment of AIDS. The Pan American Health Organization (PAHO) and NIAID convened a Workshop on AIDS in the Americas held in Washington in early August. Many other meetings on AIDS were held with scientists, public health officials, and public interest groups.

Lectures, speaking engagements, and publications of the Director included:

"The Beginning of Health is to Know the Disease," November 8, 1982, presented at the Thirty-first Annual Meeting of the American Society of Tropical Medicine and Hygiene, Cleveland, Ohio.

"Malaria, the King of Diseases," November 15, 1982, presented at the Basel Institute for Immunology, Basel, Switzerland.

Introduction of Senator Mark O. Hatfield, March 20, 1983, at Annual Meeting of the American Academy of Allergy and Immunology (AAAI), Hollywood, Florida.

"Acquired Immune Deficiency Syndrome," (AIDS), April 12-13, 1983, Stone House, NIH, Bethesda, Maryland.

Report of the Director, NIAID, to National Digestive Diseases Advisory Board, April 27, 1983, Linden Hill Hotel, Bethesda, Maryland.

"History and the Allergic Diseases," April 30, 1983, Asthma and Other Allergic Diseases, Management by Primary Physicians, Northwestern University Medical School, Chicago, Illinois.

"AIDS as Seen Through the Prism of History," June 21, 1983, presented at the University of Alabama, Birmingham.

"AIDS as Seen Through the Prism of History," August 1, 1983, presented at International Society for STD Research, 5th Annual Meeting in Seattle, Washington.

"Koch's Postulates in the Search for the AIDS Agent," August 24, 1983, presented at the First International Congress for Infectious Diseases, Vienna, Austria.

"AIDS as Seen Through the Prism of History," September 19, 1983 presented at the Second Annual Conference for Science Writers co-sponsored by the American Medical Association and Northwestern University, Chicago, Illinois.

"Is the Biological Revolution a Match for the Trinity of Despair?" published in Fall 1983 issue of Technology and Society.

"The Beginning of Health is to Know the Disease," to be published in Fall 1983 issue of Public Health Reports.

"The Complete Book of Vaccines," to be published in November 1983 issue of Good Housekeeping.

Contributed chapter on Streptococcal Diseases for next edition of Cecil's Textbook of Medicine.

The Director serves, both nationally and internationally, as a consultant and advisor on a broad range of health matters. Recently, he was appointed a member of the Scientific Advisory Committee of the Max-Planck Institute for Immunobiology in Freiburg, and continues as Director of the Biomedical Sciences and Technical Review Committee (BIOS) of the World Health Organization (WHO). He is a consultant and member of the Coccal Expert Committee, WHO, and consultant to the Surgeon General of the U.S. Army. He has also served, since 1977, as a Member of the Albert Lasker Medical Research Awards Jury.

In February of 1983, Dr. Krause participated in the first Indo-U.S. Senior Scientific Panel for collaboration in science and technology between the United States and India. The Panel was established under an agreement reached by Indian Prime Minister Gandhi and U.S. President Reagan to increase cooperation between the two countries. Dr. Krause was one of a six-member delegation of U.S. scientists chosen to meet with a team of Indian scientists to identify major areas in which effective collaboration could take place. The five areas

selected were agriculture, health, human fertility, earth sciences, and material sciences. In the health field, the team chose these initial cooperative projects: (1) application of immunologic advances in the diagnosis and prevention of infectious diseases, such as leprosy and filariasis, (2) control of blindness, with emphasis on cataracts, and (3) immunologic approaches to fertility regulation and infertility.

Later this year, the Director and NIAID scientists again met with Indian scientists who visited the National Institutes of Health to further discuss joint research in the designated areas. They also discussed a potential link between U.S.-India Workshops and the U.S.-Japan Panel Meetings. During these discussions several other diseases were selected for special emphasis: tuberculosis, malaria and Japanese B encephalitis.

Dr. Bernard Talbot, the Deputy Director of the Institute, testified before Congressional committees five times this fiscal year. In addition to accompanying Dr. Krause to testify before the Senate (April 12, 1983) and House (April 25, 1983) Subcommittees on Appropriations on the FY 1984 NIAID budget, he also testified on:

November 18, 1982, before House Subcommittee on Investigations and Oversight (Albert Gore, Jr., Chairman), Committee on Science and Technology, in hearing on "Human Applications of Genetic Engineering."

May 9, 1983, before House Subcommittee on Health and the Environment (Henry A. Waxman, Chairman), Committee on Energy and Commerce, in hearing on H.R. 2713 and Related Legislation -Public Health Emergency Response.

June 22, 1983, before House Subcommittee on Science, Research and Technology (Doug Walgren, Chairman), and Subcommittee on Investigations and Oversight (Albert Gore, Jr., Chairman), Committee on Science and Technology, in hearing on "Environmental Implications of Genetic Engineering."

Lectures, talks, and publications of the Deputy Director included:

"Congressional Hearing on Human Applications of Genetic Engineering" Published in March 1983 issue of Recombinant DNA Technical Bulletin.

"Development of the National Institutes of Health Guidelines for Recombinant DNA Research," published in the July-August 1983 issue of Public Health Reports.

Introductory remarks at the U.S-Japan Cooperative Program for Recombinant DNA Research Workshop on Gene Transfer in Eukaryotic Cells, San Diego, California, November 8, 1982.

Talk at Annual Meeting of Association of Medical School Microbiology Chairmen, Santa Barbara, California, January 22, 1983.

Talk at meeting of Public and Scientific Affairs Board, American Society for Microbiology, New Orleans, Louisiana, March 7, 1983.

Talk at Round Table "Current Issues in Biomedical Ethics" at Annual Meeting, American Society for Microbiology, New Orleans, Louisiana, March 9, 1983.

Introductory remarks at U.S.-Japan Cooperative Program for Recombinant DNA Research Workshop on Medical Applications of Recombinant DNA Research, Honolulu, Hawaii, May 26, 1983.

In July, the Institute lost one of its most distinguished scientists. Dr. Wallace P. Rowe, who had been chief of the Laboratory of Viral Diseases since 1968, succumbed to cancer at age 57. One of the world's leading virologists, Dr. Rowe made significant contributions to medical science beginning with his discovery of a new group of viruses--known as adenoviruses--to his most recent studies of the natural history of murine retrovirus infection.

The following honors and awards were received by NIAID scientists: former Deputy Director of NIAID, Dr. John R. Seal, was elected to Senior Membership in the Institute of Medicine of the National Academy of Sciences; Dr. George Galasso received the Assistant Secretary for Health's Award for Exceptional Achievement; Dr. Stephen E. Straus and Dr. Karl Western were awarded PHS Commendation Medals; Dr. Albert Kapikian was presented PHS's highest honor--the Distinguished Service Medal; the PHS Superior Service Award was given to Dr. Louis Miller; Dr. Hilton Levy received the Inventor's Award given by the National Technical Information Service, Department of Commerce; Dr. Yasutaka Hoshino received the Ralston Purina Small Animal Research Award; and SES bonuses for 1982 were given to Drs. Franklin Neva, Kenneth Sell, and Thomas Kindt.

Office of Program Planning and Evaluation

During the Fiscal Year 1983, this office continued to serve the Director as advisor on the development, analysis, and evaluation of the Institute's programs; provided support and liaison to program managers on coordinating, integrating, and articulating long-range program goals and strategies; and served as an advisor on legislative and administrative developments which included projecting their impact on NIAID programs and operations. The major documents for which the OPPE had lead responsibility were the Institute's Annual Research and Evaluation Plans and associated reports. It also responded to requests from trans-NIH committees and other sources for program information and assisted in preparing material required for the Congressional budget presentations, NIH, and HHS reports. The Office has responsibility for scientifically classifying and categorizing all research projects supported by the Institute and maintaining the computerized system for recording such information.

Office of Recombinant DNA Activities

The NIH Guidelines for Research Involving Recombinant DNA Molecules continued to evolve as major actions recommended by the Recombinant DNA Advisory Committee (RAC) were approved by the Director, NIAID. The RAC met on October 25, 1982 and April 11, 1983, and will meet on September 19, 1983. Major actions under the Guidelines were published in the Federal Register on January 10, April 15, and June 1, 1983. A major revision of the Guidelines was promulgated in the Federal Register on June 1, 1983. In this revision, a new Appendix L has been

added which defines conditions under which a working group of the RAC can approve release into the environment of certain genetically engineered plants. Also, the Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules, which had been published separately, were formally incorporated into the Guidelines as Appendix K. A Working Group was established to prepare a response to a report on the Social and Ethical Issues of Genetic Engineering with Human Beings which was prepared by the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research. This Working Group met on June 24, 1983, and made recommendations on RAC's possible role in this area. These recommendations will be considered by the full RAC at its meeting on September 19, 1983. Procedures for the handling of confidential information were reinstated in July 1983 in response to requests from private industry for review of proposals involving proprietary information.

ORDA continued to interact with the international scientific community during FY 83. Under the U.S.-Japan Cooperative Program for Recombinant DNA Research, ORDA hosted a Workshop on Gene Transfer in Eukaryotic Cells in San Diego on November 8-9, 1982. ORDA also hosted a Workshop on Medical Applications of Recombinant DNA Research in Honolulu on May 26-27, 1983. The latter workshop was also the occasion of the third U.S.-Japan bilateral meeting which resulted in the signing of an updated Memorandum of Understanding. The Director, ORDA, served as chairman of a group of experts which met at the headquarters of the Organization for Economic Cooperation and Development (OECD) on December 9, 1982, to provide guidance for an OECD study on safety and regulations in biotechnology. Subsequently, the Director, ORDA, was nominated to be the U.S. representative to the Group of Government Experts on Safety and Regulations in Biotechnology which will carry out this study. The Director, ORDA, attended the annual meeting of the Committee on Genetic Experimentation (COGENE) of the International Council of Scientific Unions at the University of Cologne on April 6, 1983. Although the U.S. is not a participant, ORDA has followed closely developments in the planning for an International Center for Genetic Engineering and Biotechnology by the United Nations Industrial Development Organization (UNIDO). The Director, ORDA, provided an overview of the proposed UNIDO Center at a symposium at the Foreign Service Institute of the U.S. Department of State on September 8, 1983.

ORDA presented information on recombinant DNA research and the NIH Guidelines at a symposium on "DNA Research: Commercial and Legal Aspects" at the Waksman Institute of Microbiology in New Brunswick, New Jersey, on December 3, 1982, and at a course on "Science and the Law: Biotechnology" sponsored by the Foundation for Advanced Education in the Sciences at NIH on April 27, 1983. The Director, ORDA, prepared a paper on "Cooperative Efforts in Development of Safety Guidelines for Recombinant DNA Research," for a meeting on U.S. participation in international science and technology cooperation sponsored by the National Research Council on September 28-29, 1983. Within the NIH community, ORDA provided orientation talks on the NIH Guidelines and NIH policies to the new members of the Extramural Associates Program on February 3 and July 28, 1983.

Office of Research Reporting and Public Response

Fiscal Year 1983 brought major changes to the NIAID Office of Research Reporting and Public Response (ORRPR). Former Chief of the Office, Robert L.

Schreiber, left NIAID. Dr. Jack Whitescarver assumed the role of Acting Chief of ORRPR (while Nancy Brun was given the responsibility for day-to-day technical supervision) until a new permanent Chief is appointed. Marian McGrath joined the staff as a technical publications writer-editor.

Technical editorial assistance was provided by ORRPR to the NIAID director on his speeches as well as on his other writing projects. The staff also gave editorial and technical assistance on two publications on the immune system. A shorter version, The Immune System, was published in May as a cooperative project with the Asthma & Allergy Foundation of America (AAFA). A longer version, intended for science writers and some health professionals, will be published next year.

Several important new initiatives were undertaken by ORRPR, including a rabies public education campaign, a planned information campaign about toxoplasmosis, a campaign to give wider distribution to NIAID publications, and one to strengthen ties between NIAID in Bethesda and the Rocky Mountain Laboratories and its community in Hamilton, Montana.

In a first-of-its-kind cooperative effort, ORRPR worked closely with Giant Foods Incorporated, Metrobus, D.C. Medical Society and Wyeth Laboratories to launch a major campaign to alert area residents and health professionals about the current rabies epidemic in the Washington Metropolitan area. The theme was how people can protect themselves against rabies. Camera-ready copy was provided to Giant for a public service message that was printed on 100,000 Giant-brand half-gallon milk cartons, and as an insert in the Company's pharmacy advertisement that appeared in the July 27 issue of the Washington Post. As a public service, Metrobus mounted 200 six-foot "messages" on the backs of buses for a nine-month period. To meet the potential demand for information generated by this publicity Wyeth Laboratories, manufacturers of Wyvac human rabies vaccine, reprinted 50,000 copies of the rabies fact sheet free of charge. In addition to help from these groups, the Medical Society of the District of Columbia reprinted ORRPR's fact sheet on rabies as part of its July 1983 newsletter which is distributed to 3,400 physicians in the District of Columbia.

In the planning stage is a campaign on toxoplasmosis, a topic of vital importance to pregnant women and to those planning to become pregnant. Initial efforts are underway to develop TV and radio spots, articles and features for popular women's magazines and NIH publications, and to print the newly revised fact sheet on toxoplasmosis. This material will alert women to the dangers of contracting toxoplasmosis during pregnancy, which can result in severe damage or death to newborns.

Closer liaison with the NIAID Rocky Mountain Laboratories (RML) in Hamilton, Montana, is a new goal of the ORRPR. Two ORRPR staff members visited RML in July to plan and initiate this liaison and to increase Hamilton community awareness of NIAID/NIH and its relation to RML. Exhibits will be shared and NIAID publications will be widely distributed in western Montana. The local media has already begun increased coverage of the work of RML scientists and Montana health groups are organizing professional education programs using NIAID pamphlets and films.

NIAID's film, Jennifer: A Revealing Story About Genital Herpes, continues to be in great demand with approximately 5,000 bookings scheduled for this fiscal year. The film has been seen by more than a half-million people throughout the United States and Canada this year and well over one million for the two years it has been in distribution. It has also been shown on cable and commercial TV to over a half million viewers this fiscal year, and almost one and a half million since the start of distribution in March 1982. ORRPR staff updated the film adding information about FDA's licensing of an ointment form of the drug acyclovir, for the treatment of primary genital herpes.

Production of a new slide-tape show, "Coping with Your Allergies, At Home, At School, and On the Job" was completed this year. Although available only since April, approximately 50 copies have already been sold. ORRPR plans to make this educational tool available to health professionals and interested voluntary groups in the U.S.

The epidemic of Acquired Immune Deficiency Syndrome (AIDS) has heightened activity in the ORRPR with a substantial increase in requests for information by telephone and letter, as well as in press activity. Because of repeated demands for TV coverage of the Institute's AIDS research facilities, ORRPR and NIH staff developed a 25-minute videotape showing the clinical and laboratory work currently being done by Dr. Anthony Fauci and his colleagues in the Laboratory of Immunoregulation. The footage has been used repeatedly by TV stations, both locally and nationally.

Inquiries for information and/or interviews were received from nearly 1,000 media representatives. In addition to AIDS, the media expressed special interest in allergic diseases, genital herpes, Lyme Disease, whose causative agent was discovered this year by scientists at the NIAID Rocky Mountain Laboratories as well as in many other diseases currently under study by the Institute. Research Reports were prepared and sent to writers and editors across the U.S. on timely topics such as the hepatitis B vaccine, confirmation of the cause of Lyme Disease, and vaccinia virus recombinants. Parke-Davis cooperated on a story about the beneficial use of vidarabine in treating immunosuppressed patients who develop herpes zoster. The drug was approved by the FDA in May for this use. The staff also prepared press releases to announce new grants supporting AIDS research, a contract to develop an improved pertussis vaccine, funding of a new sexually transmitted diseases research center, and other selected research advances.

A new table-top exhibit was purchased by the ORRPR and used initially at the Family Conference on Asthma and Allergies held at Howard University. It has also been used at various cluster meetings and health fairs in the local area. This versatile exhibit will easily lend itself to a variety of themes that can be developed as meetings are scheduled for the future.

NIAID exhibited at the American Academy of Allergy meeting in Hollywood, Florida, the Lupus Meeting in the District of Columbia, and as an invited participant at the Mid-Atlantic Regional Patient Education Symposium hosted by Pennsylvania State University at Hershey, Pennsylvania. ORRPR staff also assisted in shipping its large freestanding allergy exhibit, as well as in providing publications, for the National Medical Association Meeting in Chicago.

A special report on sexually transmitted diseases was prepared by ORRPR staff for the Senate and House Appropriations subcommittees. Six additional "mini-reports" were also prepared on NIAID's research related to arthritis, Alzheimer's disease, cystic fibrosis, diabetes, digestive diseases, and genetics.

Seven articles were written for NIH's News and Features, and 10 articles were written for the NIH Search for Health Columns, both of which are sent to a broad media audience. Nearly 35 stories on Institute activities were published in the NIH Record. ORRPR staff reviewed approximately 500 manuscripts prepared by Institute scientists and administrators for publication in professional journals or to be presented at scientific meetings. ORRPR staff responded to approximately 600 letters and more than 3,200 telephone calls from the public. Congressional telephone and letter requests totaled nearly 50. Approximately 75 Freedom of Information requests were handled.

Publications printed during this fiscal year were: The Immune System, Dust Allergy, Sinusitis, and Rabies. Well over a quarter of a million publications were distributed by ORRPR staff this year. Included in this total were bulk copies furnished for exhibits, health fairs, workshops, and other professional meetings. In an effort to increase the availability of Institute publications, ORRPR reached an agreement with the Government Printing Office to add four more publications--increasing the total number to six--to their list of items that can be purchased in bulk quantities. ORRPR also obtained free advertising space in two medical journals--Journal of the National Medical Association and Journal of Family Practice-- to announce the availability in bulk of these popular brochures. This should be helpful to physicians and clinics who wish to distribute the Institute's patient education material.

ORRPR continues its close ties with organizations the Asthma & Allergy Foundation of America (AAFA), The Lupus Foundation of America (LFA) and the National Foundation for Infectious Diseases (NFID). ORRPR staff met early this year with representatives of AAFA and NFID to discuss ways to work together to accomplish our common goals of educating the public about allergic and infectious diseases. ORRPR cooperated with these organizations on several outreach public and professional symposia and workshops on lupus and on asthma and allergies. Local meetings of the Metropolitan Washington Chapter of AAFA are attended by ORRPR staff, and publications on allergies and on asthma are furnished for distribution at their meetings.

Annual Report
Immunology, Allergic and Immunologic Diseases Program
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REPORT OF THE DIRECTOR
IMMUNOLOGY, ALLERGIC AND IMMUNOLOGIC DISEASES PROGRAM
Fiscal Year 1983

A. Administrative Summary

1. Organization and Functions

The Immunology, Allergic and Immunologic Diseases Program (IAIDP) was initiated on October 1, 1976, coincident with the reorganization of NIAID into major programs characterized by special professional functions. With this change the IAIDP assumed the responsibility for research, training, and conference activities that formerly were assigned to the Allergy and Immunology Branch of the Extramural Programs and contract activities in transplantation immunology and immunologic research resources previously administered within the Collaborative Research Program.

During FY 1983, the IAID Program operated according to the following organizational plan:

Office of the Director (OD)

Director-Sheldon G. Cohen, M.D.
Deputy Director-Bernard W. Janicki, Ph.D.
Special Assistant to the Director-Dorothy D. Sogn, M.D.
Program Officer-Judith G. Massicot, Ph.D.
Program Analyst-Charles Hamilton

Clinical Applications Section (CAS)
Head-Daniel I. Mullally, M.D., M.P.H.

Allergy and Clinical Immunology Branch (ACIB)

Chief-Robert A. Goldstein, M.D., Ph.D.
Program Associate-Janet Ayres

Immunology and Immunochemistry Branch (IIB)

Chief-Bernard W. Janicki, Ph.D.
Program Officer-John G. Ray, Jr., Ph.D.

Genetics and Transplantation Biology Branch (GTBB)

Chief, Henry Krakauer, M.D., Ph.D. (10/1/82-1/1/83)
Acting Chief, Daniel I. Mullally, M.D., M.P.H. (1/1/83-9/30/83)
Acting Assistant Chief-John F. Finerty, Ph.D. (1/1/83-9/30/83)
Program Associate-Justina S. Grauman, M.S.
Serum Bank Manager-Anne Heath

Each of the three IAIDP Branches assumes responsibility for administering initiated research grants and collaborative research contract awards, developing programmatic initiatives and new projects, serving on trans-NIH coordinating committees and taking active roles in the development and conduct of workshops

and conferences in areas of biomedical and biological disciplines and the clinical medical specialities with which they are concerned.

All three Branches are also involved with programs related to Research Career Development Awards and training, i.e., individual and institutional Fellowships and the Young Investigator Research Grant Awards designed to assist and encourage investigators in early stages of their careers to develop research interests and capabilities in immunology. In addition, the Allergy and Clinical Immunology Branch administers Allergic Disease Academic Awards which are designed to further the careers of mid-level investigators on career tracks in academic and research allergy, and this year initiated the Clinical Investigator Award. The purpose of this new program is to provide opportunities for young individuals trained as clinicians with demonstrable interest and aptitude in the fields of immunology and allergy, and in turn to increase the pool of clinical investigators applying knowledge and relevant basic biomedical sciences to clinical problems in allergic and immunologic diseases.

The IAID Program also assists investigators by procuring and providing certain reference standard and research reagents not otherwise available from commercial sources. These have included human and mouse histocompatibility typing sera, purified allergens, and antisera to lymphocyte subsets. Program staff's responsibility and awareness of needs to assist constituent fields are reflected in the expansion and development of several new endeavors in this area.

2. Centers and Program Projects

The IAIDP/NIAID Clinical Research Centers Program is now comprised of 28 centers.

Sixteen Asthma and Allergic Disease Centers located at: Scripps Clinic and Research Foundation, Mayo Clinic, and NIAID NIH Clinical Center, and university medical school and hospital centers at California/San Francisco, Northwestern, Tulane, Harvard/Brigham and Women's, SUNY/Stoney Brook, Duke, Texas/Dallas, Wisconsin, Tufts, UC San Diego, Rockefeller-Cornell University Medical Center, Medical College of Wisconsin, and the recently approved center at the University of Iowa.

The six centers for Interdisciplinary Research in Immunologic Diseases are located at: UCLA, Georgetown, Washington (St. Louis), Rochester, N.Y., Johns Hopkins and Children's Hospital, Boston. In addition to the conduct of basic biomedical research and clinical investigations in asthma, allergic and immunologic diseases and the application of basic data to attacking clinical problems in diagnosis, prevention and treatment of patients with these disorders through multi-disciplinary collaborative approaches, these centers have been focusing efforts on the development of educational and community activities, outreach regional programs for both professional groups and the laity.

To encourage investigations of the underlying mechanisms of immunologic disease and to enhance basic knowledge relevant to the etiology, prevention and management of such disorders effective from one of two disciplinary approaches, clinical immunology or immunopathology, the ACIB has established a special program, Mechanisms of Immunologic Diseases. Six program projects were operational

at university medical school and hospital centers at Scripps (2), Stanford, University of Alabama, Tufts and U.C. San Francisco.

Through the provisions of the Lymphocyte Biology Program the IIB has set the goal of attaining comprehensive knowledge of the life history of immunocompetent cells and of the genetic and phenotypic factors that determine cellular fate and function in vivo and in vitro. Program project studies (Lymphocyte Biology Centers) in this defined area are in progress at seven university and medical centers located at the Rockefeller University, Yeshiva University, University of Texas (Dallas), Harvard Medical School, Jewish Hospital of St. Louis, Stanford University, and the Scripps Clinic and Research Foundation. During this fiscal year, the Rockefeller and Harvard Centers successfully competed for renewal awards and the centers at Stanford and Scripps were newly established.

In supporting projects in transplantation immunology, the objective of the GTBB is to take advantage of the momentous advances in technical procedures and in the understanding of the molecular cellular processes to bypass remaining hazards associated with immunosuppressive therapy and rejection of organ transplants. Focus is given to the application of the most up-to-date concepts and techniques of immunology to the evaluation of the immune system of recipients in all circumstances attendant to transplantation. Of especial concern is clarification of immunoregulatory balance and modulation of immunologic activity. The first Program project in this area was awarded to Johns Hopkins Medical School and Hospital. Subsequently two additional Program Projects were initiated at Duke University Medical Center and the Brigham and Women's Hospital (Harvard Medical School).

By annual announcements (RFA) new institutions are invited to compete for available funds with ongoing Centers at the time of submission of renewal applications. An additional feature of this Centers - Program Project endeavor has been inter-Branch facilitation and support of investigator exchanges between institutions comprising this network. Rapid developments and progress in research on physiologic function, chemistry and the genetics of the immunocompetent cells have not only been rewarding in revealing fundamental knowledge but additionally have pointed to relevant leads for effecting clinical relevance. Accordingly, staff exchanges are designed to favor applications of basic biomedical science data to clinical investigations.

3. Special Research Grant Announcements

During FY 1983 IAIDP issued the following Requests For Research Grant Applications (RFA):

- a. To allow for continuation of ongoing programs described above.

Asthma and Allergic Disease Centers
Projects in Mechanisms of Immunologic Disease
Program Projects in Lymphocyte Biology

- b. ACIB sponsored joint Institute Announcements for cooperative research initiatives:

ACIB-sponsored joint Institute Announcements for research initiatives on: Immunopathogenic Mechanisms Involved in the Development of Insulin-Dependent Diabetes Mellitus (IDDM), with NIADDK and NICHD; Immune Mechanisms of Cutaneous Disorders, Immunodermatology, with NIADDK.

NIAID, with NIADDK, will recruit physicians trained in clinical endocrinology for placement in NIAID supported immunology postdoctoral training programs in order to prepare them for research careers in the immunologic and immunopathologic aspects of diabetes.

NIAID is collaborating with NIADDK in initiating within that Institute's Special Emphasis Research Career Award (SERCA) program an award focused on immunologic factors in diabetes mellitus. This award provides the opportunity for a physician with developing research interests to acquire multidisciplinary experience in basic and clinical areas needed to study the metabolic, endocrinologic and immunologic aspects of diabetes mellitus.

A new award mechanism was begun in FY83, the Physician Scientist Award. The award is intended to encourage the individual with clinical training to develop research skills in a fundamental science. To help support the transition the awards will enable physicians to undertake up to five years of special study in basic science with a supervised research experience. The first phase will include both didactic study and laboratory experience conducted under the close sponsorship of an individual with extensive research experience. The second phase under the continuing guidance of this primary sponsor, will be to apply laboratory based research in either a basic science or clinical department.

4. Collaborative Projects

The Hybridoma Cell Line Bank contract awarded to the American Type Culture Collection has continued the distribution and propagation stages, and is continuing with additional acquisitions. 281 shipments of cell lines have been sent to 260 investigators; 82 cell lines are available now for distribution; 14 cell lines are being processed and 11 lines are being held pending further study for usefulness.

The ACIB continued developing its research capabilities by completing the acquisition, storage and distribution of reference standard materials which include: a battery of fungal antigens as well as human antisera relating to the identification of etiologic factors of hypersensitivity in pneumonitis, selected lyophilized preparations of ten food materials for standardized skin tests as well as oral challenge studies, a complement standard (C3) for biological assays, an antihuman IgG-horseradish peroxidase agent, as well as short ragweed and antigen E and K for research studies in allergy. Thus ACIB effort by quality control is supported by a contract awarded to the Mayo Foundation for operation of an Allergen Resource References Laboratory.

By contract support the GTBB has continued to assist in the work of the International Bone Marrow Transplantation Registry maintained at the Mt. Sinai Medical Center in Milwaukee. Data collection is designed to provide pertinent information on immune processes and immunologic problems associated with bone marrow transplantation and thus represents a unique resource to the medical science and clinical communities coming under the GTBB's purview.

The clinical trial of skin testing with major and minor penicillin derivatives in hospitalized adults is now being extended into its fourth year. Reagents are available for testing at each of 7 participating university medical school and hospital centers located at Washington (Seattle), Northwestern, Colorado, Rochester, (N.Y.) Cornell, Walter Reed Army Medical Center and the Clinical Center NIAID-NIH. At issue is whether skin testing with major and minor determinants of penicillin can predict hypersensitivity reactions to administration of penicillin (or its analogs) in history positive and in history negative hospitalized adults. If this predictive value is shown, the study may further demonstrate whether a positive or negative history adds information in this respect, beyond that gleaned from skin tests. The required number of history positive patients has been exceeded. 750 have thus far been enrolled. 77% of such patients are skin test negative and may receive penicillin without danger of immediate life-threatening allergic reactions. Of the required 3500 history negative patients, 604 patients have thus far been enrolled. 3% are skin test positive leading to the assumption that such patients are at high risk for immediate life-threatening allergic reactions to penicillin.

Renewal support was given for continuation of the trans-NIH contract project on tabulation and analysis of immunoglobulin sequence data under the direction of Dr. Elvin Kabat, who joined the NIAID last year in the capacity of Expert (immunochemistry). The object of this project is to publish sequence data into a single reference resource, to develop further concepts concerning immunoglobulin synthesis and diversity, and to expand the data base to include nucleic acid sequences of immunoglobulin chains and the inclusion of sequence data on HLA, H-2, Ia, and related molecules.

A collaborative study at the Sidney Farber Cancer Institute is concerned with investigation on immunodepressant effects of a series of conventional drugs including corticosteroids, cytotoxic agents, antilymphocyte/thymocyte serum or globulin and total lymphoid irradiation. Indications have been obtained that some of these agents do selectively depress subsets of T lymphocytes. This can serve as the first step in the elucidation of the mechanisms of action of these conventional immunodepressant drugs and agents. In addition these data provide the insights that will be needed for the proper employment of subset specific monoclonal antibodies as immunomodulating agents. In conjunction with certain pilot studies on the use of these agents being conducted presently, within a very short time it may be possible to conduct a full scale clinical trial of these monoclonal antibodies in transplantation and in a variety of autoimmune disorders.

Requests for Proposals (RFP)

During 1983 the following RFPs were either issued or responses reviewed in order to initiate new projects and further ongoing work in identified areas of need and to invite competition for awards for ongoing work at the time of submission of renewal applicant proposals.

Maintenance and continued development of the NIAID initiated HLA serum bank; so that the complement of reagents that have come to be considered reference standards might continue to be available for quality assurance programs; to make available low cost reagents to investigators not involved in any cost reimbursable clinical activity.

Request for contracts to effect Master Agreements concerned with the development and supply of serologic reagents for the investigation of polymorphic human cell surface antigens was announced.

5. Research Resources

The IIB has provided qualified researchers with the highest possible specificity and purity antisera to available cellular antigenic determinants for basic immunologic investigations of mouse systems: Ia, Ly, H-2, Thy-1, Qa-1, Lna, PC.1 and T1. Support of this programmatic interest is a development of the recognition of the role of murine strains as the focal point of research in experimental immunogenetics because of the effect of this series of closely linked genes on tissue transplantation, susceptibility to viral infection and cell interactions during immune responses. 35 ml. were distributed to 19 qualified investigators, in 12 shipments. There are 19 new antisera ready for distribution, 11 under development and 8 in the exploratory stage.

A newly developed immunologic H-2 complex reagent has been made available to grantees and qualified investigators studying manipulation of the immune mechanisms by antigen recognition control and immunotherapy through the work of the IIB. Newer specificities of H-2 antisera related to tissue histocompatibility and specific Ia antisera involved in immune response mechanisms have been identified. Ly antisera specific for thymus dependent lymphocytes and for non-histocompatibility cell marker studies, tumor specific antibodies and theta cell antisera have been developed, banked and distributed. 1186 vials of alloantisera were distributed to 95 qualified investigators in 97 U.S. and 31 foreign shipments.

A manual comprising various tissue typing techniques, methods for freezing tissue cells and the acquisition of antisera is available and has been provided to qualified researchers and medical libraries.

Contracts are in place for:

Preparation of alloantisera specific for H-2 murine cell membrane antigens: To provide a continuous source of well characterized, functionally active H-2 alloantisera for distribution to qualified investigators and for quality control testing of other acquired antisera; awarded to Mayo Foundation.

Development of methods to prepare bulk supplies of alloantisera specific for mouse cell surface determinants. To continue the development of methodology for the production of alloantisera specific mouse cell surface antigens, the preparation of relatively small quantities of such antisera, the storage of these reagents and their distribution to selected qualified investigators, and the provision of quality control testing of other acquired antisera; awarded to Sloan-Kettering Institute for Cancer Research.

Screening, Characterization, and Characterization of Histocompatibility Testing Antisera Capable of a More Effective Definition of Transplantation Antigens of the Ethnic Subpopulations in the United States: To overcome difficulties in tissue typing of noncaucasians, an outcome that is unsatisfactory from the theoretical immunogenetics standpoint and in terms of its practical consequences in organ and tissue transplantation; awarded to Georgetown University and the Sloan Kettering Institute and Columbia University New York.

Development and clinical evaluation of a rapid cellular cross matching technique suitable for use in cadaveric organ transplantation. To develop a cellular cross matching technique sufficiently rapid to be useful in estimating histocompatibility between recipient and cadaveric donors in renal and other organ transplantation and to supplement incomplete information determined by serologic cross matching for free formed antibodies; awarded to the Brigham and Women's Hospital (Harvard Medical School).

Allergen resource reference laboratory. To acquire and make available standardized materials, allergens and other immunopharmacologic reagents, for clinical investigations and to serve as a reference laboratory for the production, analysis, assay and quality control of a variety of immunologic-allergenic reagents and materials and as a centralized reference laboratory facility for immunodiagnostic tests; awarded to the Allergy Research Laboratory of the Mayo Foundation.

6. Histocompatibility Serum Bank Activities

The inventory and ordering procedures for bank sera and the evaluation of reference laboratory reports is functioning on an automated system. The work of the Serum bank has involved 270 sera offers, 82 of which represented rare specificities, and 560 users. Seven liters of excellent reagents were acquired during this year.

A data acquisition and analysis capability in the Serum bank has been developed for the purpose of conducting inhouse studies on pooled national data in the area of immunogenetics. Preliminary analysis of this data has begun.

The policies for the distribution of histocompatibility reagents by the Serum bank have been revised in response to regulatory changes concerning the licensure of these materials. Approximately 80,000 typing trays were distributed in the past year, but the production and shipment of these trays has now been halted. Bulk reagent distribution is now limited to research and proficiency testing applications.

7. Outreach and Community Activities Program

Within the research framework and resources of the NIAID supported Asthma, Allergic and Immunologic Diseases Centers (AACD's and CIRID's), the IAID Program has encouraged and fostered the development and support of outreach and community activities programs by Centers' staffs. Among the highlights resulting from this programmatic interest were the following projects:

The Rockefeller and Cornell Universities AADC, the NY Chapter of the Lupus Foundation of American (LFA) and the NIAID sponsored a public symposium: Systemic Lupus Erythematosus--A Medical Update, October 23, 1982.

The Georgetown CIRID, the Metropolitan Washington Chapter of the Asthma and Allergy Foundation of America (AAFA) and the NIAID sponsored a community conference: Asthma and Allergies, November 20, 1982.

Northwestern AADC with the Greater Chicago Chapter of AAFA sponsored a conference: Asthma and the Other Allergic Diseases, Management by Primary Care

Physicians; and a Community forum: The Best in Research, Resources and Relief, April 30, 1983.

Public programs and/or physician education programs were also held at Howard University (May 21, 1983) and UCLA (June, 1983)

Plans were formulated during FY83 for public programs to be held at the University of California San Francisco (UCSF) and the University of Southern California and Los Angeles County Hospital Center with physician education programs to be held at the Stanford and UCLA Centers for "Lupus Awareness Week" during October, 1983.

8. Awards

None

9. Special Staff Projects

Dr. Dorothy Sogn, with Dr. John Anderson of the American Academy of Allergy and Immunology (AAAI), is co-editing a monograph on Adverse Reactions to Foods. This is a joint effort of NIAID and the AAAI Research Council Committee on Adverse Reactions to Foods. The book will include sections on all types of adverse reactions with emphasis on those of known or suspected immune and/or allergic origin. Publication is scheduled for the March 1984 AAAI meeting.

The NIAID and NHLBI have funded research and development programs to teach self-management of pediatric asthma. A conference was held June 13-14, 1983 in Washington, D.C. to evaluate the state-of-the-art. The evaluation resulted in a resource document to be published as a supplement to the Journal of Allergy and Clinical Immunology.

The report of the Clinical Application Section's epidemiologic study based on a study of medical records at Children's Hospital National Medical Center of D.C. and the Prince George's County and Holy Cross Hospitals in suburban Maryland is in the process of publication. A ten fold increase has been noted over the past 25 years in the diagnosis of asthma as a leading cause of admissions among children not under continuing outpatient care. Further study will be undertaken to evaluate whether there may be demonstrable relationships to identifiable socio-economic, environmental or other etiologic and pathogenic factors to explain this phenomenon, and whether this finding may be only a local inner city phenomenon or represents a national trend.

A formal collaboration has been established between the GTBB and the Medical Information Service of the Health Care Financing Administration End Stage Renal Disease Program to evaluate data principally on transplantation contained within the files of this federal agency. This task has been substantially completed acquiring identification of a population upon whom enough information could be made available to perform the requisite analysis and definition of the date of failure of the graft. Graft and patient survival were determined as a function of age, race, sex, and primary disease. In addition similar analysis of patient survival was performed for those patients on dialysis who had not yet been transplanted. The analysis have been submitted to the Endstage Renal Disease Program and plans for further cooperative endeavor are in progress, e.g.

a mathematical model to project medical and economic impacts of change in treatment strategies on the population with chronic renal failure is being developed.

10. Liaison and Outside Activities

IAIDP Staff have joined with the Asthma and Allergy Foundation of America (AAFA) and the Lupus Foundation of America (LFA) in developing and effecting joint endeavors for educational and community outreach activities to further the causes served by these lay groups. Additionally, IAIDP staff members have been contributing to the work of professional and scientific societies and to the educational and service endeavors of medical schools and clinical institutions.

Dr. Sheldon Cohen presented the following lectures: November 8, 1982, Eastern Virginia Medical School-Norfolk, Workshop on Lupus, "Research Support in the Conquest of SLE: Role of the NIH"; November 16, 1982, Bi-Annual Symposium in Clinical Laboratory Immunology, Clearwater, Florida, "Grant Opportunities in Clinical Immunology"; March 5, 1983, Fiftieth Anniversary Sigma Xi Conference, Wilkes College, Wilkes-Barre, Pennsylvania, "Support of Biomedical Research, the Role of the NIH". On January 30, 1983 Dr. Cohen delivered a talk on "The Biological Revolution" as part of the Schering Symposium on New Horizons at the American College of Allergists in New Orleans. He was a speaker March 1, 1983 on "Eosinophils and Parasites", for the Foundation for Advanced Education in the Sciences course: Cell Biology of Immunity and Inflammation. On March 10, 1983 he spoke at a congressional briefing for members of Congress, their staffs, and representatives of national women's organizations to discuss SLE. He gave a seminar and chaired a scientific session on "Eosinophilia" at the annual meeting of the AAAI in Hollywood, Florida, March 19-23, 1983. Dr. Cohen gave an invited presentation on "The Immunologic Aspects of Eosinophilia" at the New England Society of Allergy, Boston, May 4, 1983. On April 29, 1983 he served as moderator of the Fiftieth Anniversary Health Symposium "The Politics of Medicine: Is Health Care Healthy? at Wilkes College. On June 24, 1983 he lectured on "Research Priorities of the 80's" at the Pennsylvania Allergy Association, Hershey, Pennsylvania. In Chicago, August 1, 1983 at the National Medical Association he discussed "NIAID and Allergic and Immunologic Diseases Research". On September 27, 1983 at Cornell University Medical College he spoke on "Eosinophil Biology and Chemistry". He serves on the Board of Directors and the Medical Advisory Council of the LFA and is Executive Vice President of the LFA.

Dr. Bernard Janicki presented the seminar "Primer on the NIH Grants Process for the Young Investigator" November 9, 1982, Department of Biochemistry, George Washington University Medical Center, Washington, D.C.; December 15, 1982, Department of Cell Biology, University of Texas Health Sciences Center, Dallas, Texas; February 19, 1983, Department of Pathology, University of Connecticut Health Sciences Center, Farmington, Connecticut. On October 25 1982 he addressed the Department of Biochemistry, Jefferson Medical College of Thomas Jefferson University, Philadelphia, Pennsylvania on "The Current Dilemma Facing NIH Grants". Dr. Janicki participated in the annual meeting of the American Association of Immunologists (AAI) Chicago, April 9-15, 1983 and served as a member of the AAI Travel Scholarship Committee. He served as a consultant to Dr. Gilbert Barkin, President-elect of the American Association of Certified Allergists, in the development of the program for their 1984 annual meeting. Dr. Janicki continues to chair a trans-NIH Committee on Hybridoma Issues and has

been involved in planning and developing a registry of hybridoma data. He represented the NIH group at an organizational meeting at the World Health Organization (WHO) in Geneva under the sponsorship of the Committee on Data for Science and Technology (CODATA) of the International Council of Scientific Unions. As a result of this meeting, a CODATA Task Group has been formed to develop a computer-based hybridoma data bank to serve the international research and commercial communities. Dr. Janicki has been appointed as the U.S. representative to the Task Group with other members from France, Switzerland, West Germany, Norway, England and Japan, and the World Health Organization. Multi-national funding for a three-year pilot study has been developed through the Task Group and a contract to initiate the effort has been awarded by CODATA to the American Type Culture Collection in Rockville, Maryland. Dr. Janicki has obtained complementary support for the CODATA effort from the National Dental Institute, National Institute of General Medical Sciences, National Cancer Institute, Division of Research Resources and the Bureau of Biologics. NIH support of the CODATA effort at the American Type Culture Collection is provided through the NIAID contract for a Hybridoma Cell Bank which Dr. Janicki administers. The effort is in its early developmental stages with emphasis now placed on the preparation of the questionnaires and programs for storage of data.

Dr. Robert Goldstein chaired a symposium on "Occupational Immunologic Lung Disease" (OILD) at the annual meeting of the American Association of Clinical Immunology and Allergy, November, 1982, in Anaheim, California. On November 20, 1982 he moderated a workshop at the community outreach program held at the NIH Clinical Center. In December, 1982 he convened a working group between AADC Directors and a pulmonary group concerned with aspergillosis aimed at providing centralized laboratory facilities for immunologic diagnosis. "Manpower Shortages in Academic Allergy" was the subject of an address he gave to the Program Directors at the Annual meeting of the American College of Allergists, January 1983, in New Orleans and also chaired a post-graduate session concerned with "Lung Defense Mechanisms". On February 8, 1983 he presented a lecture at the Wilmington Medical Center (Jefferson Medical College Program) on the topic of OILD. March 18-23, 1983, at the annual meeting of the American Academy of Allergy and Immunology, Dr. Goldstein addressed the workshop on Adverse Reactions to Foods concerning NIAID initiatives in this research area; co-chaired a luncheon symposium on Sarcoidosis; chaired a scientific session on Bronchial Provocation; presented material on the Wisconsin AADC's plans for a meeting on laboratory animal allergy to the AAAI Committee on Allergy to Animals; convened a meeting of the Working Group on Food Allergy to discuss scientific research protocols. May 6, 1983 he spoke at Grand Rounds, Lankenau Hospital Philadelphia, Pa. on the subject of OILD. He represented the NIAID at the meeting of the Scientific Assembly on Allergy and Clinical Immunology at the annual meeting of the American Thoracic Society held in Kansas City, May 7-10, 1983. He also served as Representative Counselor for the Scientific Assembly on Microbiology, Immunology and Infectious Diseases. At the community outreach program held at Howard University, Washington, D.C. on May 21, 1983, he co-chaired a workshop session entitled "Treatment of Asthma. On June 13-14 Dr. Goldstein co-chaired a Workshop on Research and Evaluation in Self-Management of Childhood Asthma in Washington, D.C. co-sponsored by NIAID and NHLBI. He was an invited speaker, topic: "Environmental Allergens that Cause Asthma and Other Lung Disorders," at the Southern Association of Workmen's Compensation Administrators Convention, Baltimore, June 28, 1983. An Advisory Committee was convened June 27, 1983 by

Dr. Goldstein on Occupational Allergic Diseases. The committee involved persons from academic/research, industry, labor and government, and discussed how to achieve defined goals. Additionally he serves on the faculty of the George Washington University School of Medicine and the Medical Staff of the GWU Hospital as well as the VA Hospital. He is a consultant to the Children's Hospital National Medical Center.

Dr. Dorothy Sogn gave a talk on "Allergies--An Overview" at a Department of Defense Health Seminar, November 3, 1982 and at a Gallaudet College Student Health Unit Seminar on December 1, 1982, both in Washington D.C.. She was a speaker and workshop leader (Pediatric Allergy) at a community Conference on Allergies co-sponsored by NIAID/AAFA/Georgetown CIRID held at NIH, November 20, 1982. On December 15, 1982, in Wilkes-Barre, Pennsylvania at the NPW Medical Center and on June 29, 1983 in Wheeling, West Virginia at the Ohio Valley Medical Center she gave lectures on "Clinical Applications of New Technologies in Immunology". At the AAAI annual meeting in Hollywood, Florida, she was moderator of: a symposium "Adverse Reactions to Foods"; a seminar on "Penicillin Reactions"; and a workshop on "Approaches to Diagnosis and Treatment of Drug Allergy". She is chairman of the AAAI Committee on Scientific Exhibits and Workshops and a member of the Committee on Adverse Reactions to Foods. She is co-editing a book on "Adverse Reactions to Foods" which will be a joint publication of the AAAI and the NIAID. One April 26, 1983 Dr. Sogn gave a class lecture at the University of the District of Columbia on "Diagnosis and Treatment of Allergy". She participated in the Community Conference on Allergies and Asthma co-sponsored by Howard University/NIAID/AAFA giving a talk "An Overview of Allergies and Asthma" and leading a workshop "Insect and Drug Allergy".

Dr. Henry Krakauer gave a seminar on "Management and Analysis of Data on Transplantation" at the Statistical Center of the International Bone Marrow Transplant Registry in Milwaukee on January 18, 1983. He served as a consultant to the investigators and staff of the Missouri Kidney Program, Columbia, in January 19-21, 1983 regarding immunology problems associated with kidney transplantation and therapy for end-stage renal disease. March 20-23, 1983 he was an invited speaker on the topic "Use of a Survival Model for the Assessment of Multiple Prognostic Factors in Renal Transplantation and for Cost-Effectiveness Analysis of Alternative Treatment Strategies" at the 1983 Eastern North American Region of the Biometric Society meeting in Nashville. The American Society for Artificial Internal Organs, Inc., invited Dr. Krakauer to speak at a workshop "New Biostatistical Tools in Medicine" in Toronto April 27-28, 1983.

Dr. Judith Massicot represented the IAIDP at of the Digestive Diseases Interagency Coordinating Committee on February 2, 1983 and gave a presentation on research currently sponsored by IAIDP in the area of food allergy. She served as the NIAID liaison representative to the NIH Working Group on Health and Behavior meeting February 25, 1983.

Dr. John Finerty presented a paper "Specific Isotype Responses to LS and SS Antigens of T. rhodesiense" at the FASEB meetings April 10-15, 1983 in Chicago. On March 11, 1983 he gave a seminar "Immunodeficient CBA Mice and African Trypanosomiasis: "Role of B Cells" at Howard University, Washington, D.C. He served as a consultant to the National Geographic Society to examine blood smears for parasites.

Dr. Mullally represents the NIAID on Federal interagency and trans-NIH Committees on Arthritis and on Dermatology.

11. International Health Activities

Dr. Sheldon Cohen served as moderator for a workshop on "Clinical Immunology" at the 14th Symposium of the Collegium Internationale Allergologicum, October 10-14, 1982 in Sorrento, Italy. He chaired a session of the XI International Congress of Allergology and Clinical Immunology in London October 17-22, 1982. Dr. Cohen visited St. Thomas' Hospital Medical School in London during January 1983 as a consultant for the WHO Immunology Unit to participate in the work of the WHO-IUAT Respiratory Diseases Committee on the epidemiology of asthma. He serves on the International Union of Immunological Societies (IUIS)-WHO committee on Standardization.

Drs. Sheldon Cohen, Dorothy Sogn, and Robert Goldstein have developed the questionnaire for the ongoing WHO epidemiologic study on the prevalence and nature of asthma and allergic disease in developing countries, initiated in Kuwait, Venezuela and Thailand.

Dr. Cohen continues to serve on an Immunology Advisory Committee to WHO and as consultant to the Ministry of Public Health in Kuwait for the development of an allergy center for diagnostic referrals, training and research to serve the Arabian Gulf area.

U.S. -People's Republic of China Program for Cooperation in the Science and Technology of Medicine and Public Health: IAIDP has been assisting the NIAID Director, Dr. Richard Krause, in his responsibilities as chairman for the U.S. Section on Immunology within the Joint Health Committee of the Developing U.S.-PRC Program. Specific studies for cooperative research will include HLA tissue typing and disease associations, allergy, immunity to infections and specific problems in clinical immunology. Dr. Cohen, with Dr. Kenneth Sell, led a delegation of U.S. immunologists to meet with Chinese counterparts in Beijing and Shanghai during August 1983. Discussions were held with the objective of defining possible areas where mutually supportive and cooperative investigations in allergy can be pursued.

Dr. Dorothy Sogn traveled to Caracas/Maracaibo, Venezuela as a WHO consultant to initiate an allergy survey on the epidemiology of allergy and to give an allergy lecture series at Centre Nacional de Immunologia Clinica, Maracaibo.

Dr. Bernard Janicki conducted a technical visit with Drs. Bussard and Poljak at the Pasteur Institute on January 20, 1983 in Paris. While there he presented a report on "NIH Efforts to Establish a Hybridoma Data Bank" to the CODATA Executive Committee, January 21, 1983. In Geneva, January 24-25, 1983 he participated in a meeting of the CODATA Task Group on a Hybridoma Data Bank at WHO. On February 7-9 1983 he took part in a WHO meeting on the immunology of tuberculosis at Harvard Medical School, Boston.

The third joint meeting of the U.S. and Japanese Immunology Boards was held on August 22, 1983 in Kyoto, Japan in conjunction with the Fifth International Congress of Immunology. Dr. Janicki serves as the NIAID Staff representative for the U.S. Immunology Board. At this meeting, the Board reviewed:

(1) the progress of their efforts to encourage the distribution of hybridoma cell lines and other immunologic reagents and materials in both countries and (2) the development of computer-based banks of data and information useful for investigations of the immune system. A "Workshop on Newer Technologies for Application in Immunologic Research," jointly sponsored by the Boards, was held in Hakone, Japan following the International Congress.

Dr. Robert Goldstein served as Secretary of the Subcommittee on Allergen Standardization of the IUIS and attended Executive Committee meetings in Geneva, March 1-2, 1983 and Kyoto, August 23, 1983. On March 3, 1983 he represented the NIAID at the meeting of the IUIS committee on Immunologic Reagents Standard. August 15, 1983 Dr. Goldstein gave a lecture in "Sarcoidosis and Aspergillosis" at the Japanese National Railway Hospital in Tokyo. August 18-19, 1983 he was Visiting Lecturer at the University of Kurume. August 21-27, 1983 he participated with the 5th International Congress of Immunology, Kyoto. He presented a paper at the 3rd International Paul Ehrlich Seminar, Louvain Belgium, September 18-21, 1983. While there, he served as secretary of the Executive Committee and Steering Committee of the meeting of the IUIS, Allergen Standardization Committee. He presented a paper and chaired a symposium on standardization of allergens at the 12th Congress of the European Academy of Allergology and Clinical Immunology in Rome, September 25-30, 1983. On September 29, 1983 he represented the Program at a Workshop on Allergen Standardization held in conjunction with the Congress. The Workshop involved cooperation with the Immunology Unit of the WHO and the Immunology and Allergy Section at the University of Florence.

IAIDP has continued to assist WHO in the planning and conduct of its month-long (September) annual course on Immunology of Infection for immunologists from developing countries.

IAIDP resources materials and histocompatibility sera have been provided to laboratories in foreign countries including Switzerland, France, West Germany, Netherlands, Russia, China, and Japan (Sections 5 and 6).

12. Publications

Cohen, S.G.: "The Biological Revolution" to be published in the proceedings of the Schering Symposium on New Horizons at the American College of Allergists, New Orleans, January 1983.

Cohen, S.G.: The Immunological Aspects of Eosinophilia, New England and Regional Allergy Proceedings, in press, 1983.

Cohen, S.G.: Firsts in Allergy, Boston Remembered, New England and Regional Allergy Proceedings in press, 1983.

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A monograph on "Adverse Reactions to Foods" is being jointly prepared by the NIAID and the AAAI Research Council Committee on Adverse Reactions to Foods. It will be co-edited by Dr. John Anderson of the AAAI and Dr. Dorothy Sogn of the IAIDP. It is expected to be ready for the March 1984 AAAI annual meeting.

Based upon published material in the NIAID report of the Task Force on Asthma and the Other Allergic Diseases, Program staff has worked with the AAAI Scientific and Educational Council in the development of material for a "Primer on Allergic and Immunologic Diseases" published in the Journal of the American Medical Association, volume 248, 1982.

The GTBB catalog supplement for users of histocompatibility testing reagents, including homozygous typing cells, and the Manual of Tissue Typing Techniques (revised) continue to be made available for distribution.

The ACIB has updated the catalog of available reference standards and reagents for investigations in allergy and clinical immunology entitled "NIAID Research Resources for Allergic and Immunologic Studies".

A cooperative effort with the LFA has resulted in the publication of a "Handbook for the Lupus (SLE) Patient."

13. Conferences

During FY 1983 the following meetings, conferences and workshops relevant to program activities and functions were conducted by the IAIDP.

October 23, 1982	Public Symposium: Systemic Lupus Erythematosus A Medical Update at Rockefeller University, New York, in conjunction with AADC of the Rockefeller-Cornell Universities and the NY Chapters of LFA.
November 20, 1982	Community Conference: Asthma and Allergies, NIH, Bethesda in conjunction with Metropolitan Washington Chapter of AAFA and Georgetown CIRID.
March 18, 1983	Workshop on Adverse Reactions to Foods in conjunction with the AAAI annual meeting, Hollywood Florida.
April 30, 1983	Asthma and the Other Allergic Diseases, Management by Primary Care Physicians. Also, Community Forum: The Best in Research, Resources and Relief. Co-sponsored by Greater Chicago Chapter of AAFA and the AADC at Northwestern University Medical School.

June 13-14, 1983 Research and Evaluation in Self-management of Childhood
Asthma: A Workshop, Washington, D.C., co-sponsored with
NHLBI.

June 27, 1983 Occupational Allergic Disease meeting, Chicago in conjunction with the Northwestern University Medical School AACD

Additionally IAID Program support was given to the following national and international conference and workshop endeavors:

October 17-22, 1982 11th International Congress of Allergology, London,
England

June 27-July 1, 1983 Gordon Conferences on Biological Regulatory Mechanisms,
Andover, New Hampshire

July 4-8, 1983 Gordon Conference on Phagocytes, Andover, New Hampshire

July 10-15, 1983 Research Conference on Somatic Cell Genetics, Saxtons River, Vermont

August 21-27, 1983 Fifth International Congress of Immunology, Kyoto, Japan.

14. Budget

The following shows the distribution of support by award mechanism for the activities of the Program during FY1983.

IMMUNOLOGY, ALLERGIC AND IMMUNOLOGIC DISEASES PROGRAM

FY 1983 AWARDS

<u>Award Mechanism</u>	<u>Number</u>	<u>Amount</u>
Research Grants	534	\$73,856,246
Career and Career Development Awards	<u>25</u>	<u>1,069,605</u>
Subtotal	559	\$74,925,851
Fellowship Awards	40	\$ 754,722
Training Awards	<u>25</u>	<u>2,850,424</u>
Subtotal	65	\$ 3,596,146
Contracts	<u>12</u>	<u>\$ 1,391,551</u>
Total	636	\$79,913,548

ALLERGY AND CLINICAL IMMUNOLOGY BRANCH

I. Administrative Summary

A. Scope

This Branch is concerned with the etiology, pathogenesis, diagnosis, prevention and treatment of allergic and immunologic diseases.

Studies of allergic diseases supported by the Branch include: (1) factors which contribute to asthma, such as extrinsic allergens, infections, abnormalities of the sympathetic nervous system, chemical mediators of inflammation, and pharmacologic agents; (2) immediate type hypersensitivity and its disorders (allergic rhinitis, atopic dermatitis, urticaria and angioedema); (3) allergic phenomena affecting respiratory, gastrointestinal and cutaneous tissues; (4) allergic disorders caused by insect bites and stings, foods, airborne allergens, and infectious agents; (5) immunoglobulins, particularly IgE, and the chemical mediators released by the interaction of antigen and antibody on target cells; (6) therapy and prevention of allergic disorders and hypersensitivity reactions; (7) manifestations of delayed hypersensitivity and contact dermatitis; (8) mechanisms of drug reactions and chemical sensitization; (9) isolation and chemical characterization of the active fractions of known allergenic agents; and (10) epidemiologic and environmental studies designed to ascertain those agents or substances which may be of clinical relevance for allergic individuals.

In the area of immunologic diseases, the Branch activities are focused on studies of the underlying mechanisms of disease and the application of basic knowledge to the etiology, prevention and management of immunologic disorders. Studies in clinical immunology are directed toward acquired and inherited diseases associated with dysfunctions of the immune system. Immunopathology studies include genetics, cytology, biochemistry, physiology and pharmacology of the immune system. Areas supported by the Branch include studies of (1) immune deficiency diseases arising from primary defects in development or disorders affecting immune responses, (2) clinical manifestations mediated by products of lymphocytes, (3) diseases associated with immune complexes and autoimmune phenomena, (4) immunodermatology, i.e., immune disorders involving the cutaneous system, and (5) immunotherapy of disease processes.

B. Organization

Research grant activities supported within this Branch are broadly grouped under the general programs for Allergy and Clinical Immunology. In addition, this Branch supports a Program of Institute Emphasis (PIE) for Asthma and Allergic Disease Centers, Centers for Interdisciplinary Research In Immunologic Diseases, and Program Projects In Mechanisms of Immunologic Diseases. Finally, the Branch also supports a contract activity which provides reference and research reagents and materials to facilitate ongoing work.

1. Allergy

This program is concerned with investigations of the etiologic factors, pathogenetic mechanisms, diagnostic measures, and therapeutic approaches related to the effective management of allergic disorders. In addition, the Program on Asthma is structured to investigate all of the major parameters contributing to or describing the mechanisms of causation in this disease. These include the natural history, study of allergens, bronchoprovocation challenge, animal models, pulmonary function testing, study of chemical and cell mediators, adrenergic agonists and antagonists, parasympathomimetic drugs, drug allergy, diagnostic methodology and therapeutic agents.

2. Clinical Immunology

Research grouped into this program category focuses broadly on combining and coordinating basic studies of the immune response with potential clinical relevance and application. The Program on Immunodeficiency, for example, is concerned with the etiology, ontogeny, prevention, and treatment of structural and functional deficiencies of the immune system. Both naturally occurring and acquired disease states are included. Ongoing studies have focused on the congenital absence, failure of development, or other disorders affecting thymus or bone marrow cellular elements; abnormalities in production, inhibition in metabolism of immunoglobulins; deficiencies of specific complement components; and defective host defenses due to abnormalities of leukocytes. These naturally occurring or acquired defects of immunity provide unique opportunities for scientists to expand our understanding of normal immune functions. Although some of these disorders are rare, the information gained by studying them is relevant to the general field of immunology and to many health problems including those in infectious diseases, allergy and arthritis.

Studies focused on immune complex disorders aim to clarify the role that immune complexes play in the production of disease. Immune complexes result from the binding of antigens by antibodies and arise during the course of development of the normal immune response. Under pathologic circumstances, they may appear within and harm organs and tissues. The mechanisms by which immune complexes cause tissue damage, particularly in the autoimmune diseases with which they are increasingly associated, is a subject of intense investigation.

Similarly, studies of inflammation, which is mediated by cells and humoral factors, are essential to enhance our understanding of clinical immunology. While the process of inflammation is required for host defense against foreign substances, in certain disease states inflammation plays a major role in causing tissue damage. An understanding of what initiates and therefore what modulates inflammation is crucial to understanding and controlling these disease states. The noncellular mediators of inflammation can be divided into two general categories, those of low and those of high molecular weight. The group of serum proteins derived from the complement system and the associated enzyme systems of coagulation, fibrinolysis, and kinin fall into the latter category. The initiation of the complement cascade by either the classic or alternate pathway makes a major contribution to the development of the inflammatory response. Once activated, complement components have functions including opsonization, immune adherence, chemotaxis, cell lysis, and anaphylatoxin generation.

Derangement of complement has been implicated as causing or contributing to a wide variety of disorders including cancer, kidney diseases, collagen vascular disorders, infections diseases, and inherited diseases.

3. Program of Institute Emphasis for Asthma and Allergic Disease Centers Program (AADC)

This Program currently supports a network of 15 Asthma and Allergic Disease Centers, located throughout the United States, and is actively engaged in both fundamental and applied research. Through this multifaceted approach, methods have already been developed to improve the diagnosis and treatment of asthma and other allergic diseases. Although the emphasis of the Centers Program is directed toward a better understanding of the basic mechanisms involved in the various allergic disorders, they also have provided the field of allergy with unique academic resources for the discipline of allergy. By serving as referral centers for patients in their areas, engaging in collaborative clinical trials to assess diagnostic and therapeutic discoveries, and acting as an important resource for training academic allergists for the future, they have had a profound impact on the delivery of health care to allergic individuals. Separately each of these Centers has also been successful in competing for additional support through either the Regular Grant Program, Training Grants, or Research Career Awards.

This Program of Institute Emphasis represents a major financial and philosophic commitment by the IAIDP and the NIAID to the elucidation of allergic disorders. In addition to providing support for basic science, periodic working group meetings of the Center Directors have been held, usually on an annual basis. These meetings foster continued scientific exchange as well as encourage efforts to ensure outreach activities, explore mutually beneficial community programs and other teaching methods along with establishing a network of communication among Centers, the NIAID and communities nationwide.

Renewal and new applications for this program are received on an annual basis in an open competition.

4. Program of Institute Emphasis for Centers for Interdisciplinary Research in Immunologic Diseases (CIRID)

In September of 1978, four Centers for Interdisciplinary Research in Immunologic Diseases were funded by NIAID. This Program is designed to foster integration and coordination of research projects in Clinical Immunology being pursued in clinical specialties (for example, dermatology, pulmonary medicine, hematology, nephrology, rheumatology, infectious diseases and otorhinolaryngology) with those in basic research (for example, immunochemistry, microbiology, virology, genetics, biochemistry, pharmacology, general physiology, and pathology). Furthermore, study of one or more allergic diseases is a necessary component.

An important additional component of these Centers is the funding of specific projects concerned with a variety of community activities which are designed essentially to impact on health care in a tangible and immediate way. It is hoped that these efforts along with the traditional basic

research components may provide guidance and future direction on how to best accomplish this transfer of technology.

During FY 1983 the original four Centers competed for renewal along with new applicants for continuation of this Program.

5. Program of Institute Emphasis in Mechanisms of Immunologic Diseases

Program Projects concerned with Mechanisms of Immunologic Diseases were established to encourage the development of collaborative basic science and clinical investigation in immunologic diseases. As such, this program is designed to further investigate underlying mechanisms of disease and to enhance basic knowledge relevant to etiology, prevention and management of immunologic disorders. Clinical immunology studies are directed toward acquired and inherited diseases associated with dysfunctions of the immune system. Immunopathology studies includes specific areas of genetics, cytology, biochemistry, physiology, and pharmacology of the immune system and its disorders.

This Program currently funds six Program Projects and on an annual basis renewal applicants compete with new applicants. While there is no requirement that these Program Projects include studies of allergic disorders, collaboration is encouraged among Asthma Centers, CIRIDs and Program Projects in Mechanisms of Immunologic Disorders by periodically convening working group meetings among the directors and key individual investigators.

6. Research Reference Reagents

The contract mechanism has been employed by this Program to assist current research with an emphasis on studies of allergic disorders by providing useful and unique reagents and materials to investigators. In order to aid in this process a Resource and Reference Laboratory whose function is the acquisition, quality control testing and distribution of important and unique reagents and materials has been established at the Mayo Clinic.

C. Awards and Support Levels

Relevant activities in Allergy and Clinical Immunology are supported through various mechanisms including contracts, individual post-doctoral fellowships, institutional pre- and post-doctoral training grants, career and career development awards, as well as investigator initiated research grants.

The following shows the distribution of support by award mechanisms for the activities of the Branch during FY 1983.

Allergy and Clinical Immunology Branch
FY 1983 Awards

Award Mechanism	Number	Amount*
Research Grants	210	\$ 29,585,816
Career and Career Development Awards	9	\$ 432,710
Subtotal	219	\$ 30,018,526
Fellowship Awards	9	\$ 181,771
Training Grants	17	\$ 1,819,560
Subtotal	26	\$ 2,001,331
Contracts	1	\$ 130,065
Total	246	\$ 32,149,922

*The statement for the PIE activities is included in the Research Grant Category. During FY 1983 the Branch Supported these activities with awards for 15 Asthma and Allergic Disease Centers at a total cost of \$3,459,752, six Clinical Immunology and Immunopathology Program Projects at a total cost of \$3,974,244 and six Centers for Interdisciplinary Research in Immunologic Diseases at a total cost of \$2,029,989.

II. SCIENTIFIC SUMMARY

1. Allergy

The regulation of the IgE antibody system in man provides an illustration not only of the complexity of one antibody system of great importance to gaining an understanding of allergic disorders, but also may serve to show us how to understand other complex antibody systems. The major cellular and humoral components begin with the IgE-producing B lymphocytes which may produce more (following stimulation with T helper cells) or less antibody (after suppression with T suppressor cells). In addition to cellular influences, humoral factors (suppressor and helper substances) have also been shown to be operative in regulating this immune response. Finally the IgE antibodies when formed and circulating go on to affect other defense systems of the body including tissue-fixed mast cells and circulating basophils to produce a whole panoply of chemical mediators, such as histamine, leukotrienes and serotonin.

The system itself provides a defensive mechanism, fending off certain exogenous antigens, especially those gaining access through mucoepithelial and epithelial linings such as the respiratory and gastrointestinal tract and skin. A special feature of this protection is the ability of the IgE system to be amplified in its effects beyond those ordinarily anticipated. Thus by binding in very small amounts onto the cell surface of mast cells and basophils, IgE antibody can produce much larger biological effects owing to the release of the variety of mediators mentioned (*supra vide*).

A system in delicate balance such as this appears to maintain IgE antibody production normally at a low, but adequately effective

level-probably owing to a normal "damping" effect limiting the total response to a particular stimuli. At the time of a particular perturbation one could envision the delicate balance being upset in a variety of manners. One hypothesis that many allergic disorders may occur as a consequence of an "allergic breakthrough" after appropriate stimuli. For example it has been shown in experimental animals (mice) that small doses of irradiation or immunosuppressive drugs can alter normal mechanisms of suppression sufficient to permit such animals to produce high levels of IgE.

The relatively high frequency of allergic disorders in man implies that common environmental agents may have similar effects. Respiratory viruses appear to be a candidate for just such a stimulus. Experiments in animal have shown that under certain circumstances, viral infections can induce heightened IgE responses. This has also been shown to occur in children with respiratory syncytial virus infections. It is not clear yet whether or not these individuals also had to have a genetic predilection or whether it was enough to acquire the viral infection at the right time and under the right circumstances.

Endogenous mechanisms may also affect IgE synthesis. For example, wide shifts of IgE production can be induced in experimental animals by steroids. These fluctuations could explain what happens during the course of pregnancy in allergic women, a period of improvement followed by post-partum exacerbation. Similarly such sex hormone production changes during puberty may explain the sudden disappearance of allergic disorders in many individuals who manifest those symptoms during childhood.

Interestingly it appears that once the threshold is breeched or "allergic breakthrough" has occurred the IgE production may be shown to remain elevated, instead of returning to a prestimuli baseline level. These theories may help to explain the occurrence of allergic disorders in any individual under very special circumstances. For instance the occurrence of a simple viral infection (upper respiratory) could be followed by subsequent exposure to an allergen, during a period of susceptibility. Under ordinary circumstances such an exposure would not result in any untoward effect, however the viral infection could alter the ordinary regulation of the IgE antibody response. Whether or not that would remain permanent might also require a genetic predisposition. Hereditary strains of certain mice have provided insights into the regulation of IgE antibody production. SJL mice of the low responder type can be changed to high responder animals by exposure to whole body irradiation.

Perhaps even more interesting and more exciting has been the finding that certain soluble factors in blood can be shown to alter the in vivo production of IgE antibody. The discovery of the so called "suppressive factor of allergy" led to a series of experiments which showed the ability to decrease an already elevated antibody response. Remarkably small doses were found to have a long term effect. Perhaps even more interesting was the fact that the substance influenced IgE production and not that of other antibodies. A substance with opposite effects, "enhancing factor of allergy" has also been shown to be present in the serum of both low and high responder animal models.

The demonstration that an Fc receptor specific for IgE antibody molecules exists on lymphocytes led to a series of experiments demonstrating

their presence on mast cells, basophils and eosinophils. Interestingly, exposure of normal lymphocytes to an appropriate concentration of IgE *in vitro* induces the expression of Fc receptors for IgE in these cells. Since it can be calculated that the expression of these receptors can to some extent be an indicator of total IgE concentrations in a given environment, one can easily conceive of the isotypic control of the antibody responses that would potentially become available. Cellular and humoral interactions occurring around these receptors would have the predominant regulatory influence. Experiments have recently been conducted showing that in fact the induction of such receptors can be modulated by the suppressor and enhancing factors of allergy.

Thus a considerable body of evidence now exists pertaining to the general and specific importance of the IgE antibody system in man and how it is regulated. Humans devoid of IgE have not been reported. It appears to be an important molecule; one of the first immune mechanisms to be reconstituted following bone marrow transplantation is the IgE system. Finally it should be emphasized that to whatever extent it can be determined that certain precipitating events such as common viral infection, can be shown to "cause" or permit allergic responses then prevention can occur through avoidance of these agents. In closing, knowledge of the regulation of IgE responses appears important not only for the study and control of allergic disease but serves as a model for the demonstration of an antibody response subject to delicate control and constant perturbations.

2. Clinical Immunology

In its broadest sense the study of immunodeficiency disorders has provided scientists and clinicians with much information about the normal workings of the immune system. The primary specific immunodeficiency disorders represent a group of hereditary or acquired defects. Patients affected with these diseases in general cannot mount specific immune responses and are very susceptible to infection. The primary specific immunodeficiencies all result from an absence of or maturation failure of B cells and/or T cells. Although many distinctive immunodeficiencies are now recognized only a few will be described for the purpose of this discussion.

X-linked agammaglobulinemia (congenital agammaglobulinemia or Bruton's disease). This condition is transmitted as an X-linked phenomenon. Only males are affected and these individuals have no B lymphocytes in their blood, marrow, spleen or lymph nodes. The lymphoid tissues which they do have lack germinal centers and there is a complete absence of plasma cells in lymph nodes, gut, spleen and other places where such cells are normally found. The tonsils in these children are very tiny and this comprises a major diagnostic features of the disease. On the other hand T cells are normal in number and function. The thymus gland is normal and affected individuals have normal delayed type (cell-mediated) immune responses. Because such children have protection from maternal antibody passively transferred across the placenta they have a delay in onset of clinical manifestations until age 6-9 months. Then they experience frequent infections including those of middle ear, lungs, skin and sometimes meninges. Organisms found include hemophilus influenza, diplococcus pneumonia, staph and strep. On the other hand they have no unusual susceptibility to infection with fungi, parasites, enteric bacilli and viruses. All of these latter infections tend to occur in individuals with

depressed cell-mediated immune responses. Treatment with gamma globulin can completely prevent recurrent infections and allow such individuals to grow to maturity.

Common variable agammaglobulinemia (acquired or late onset agamma globulinemia). The cause for this form of acquired deficiency is unknown. It affects both sexes and may occur at any age. In contrast to the X-linked variety, individuals do have B cell function although it tends to slowly deteriorate over a long period of time, especially in those individuals with associated tumors of the thymus gland, thymoma. Associated forms of autoimmune disorders including pernicious anemia are particularly common. Changes in B cell lining of the lamina propria of the gut may result in modular lymphoid hyperplasia which may manifest as chronic diarrhea. Treatment is with gamma globulin.

Congenital Thymic Aplasia (DiGeorge Syndrome). Though not hereditary, this condition results from an intrauterine insult to the 3rd and 4th pharyngeal pouches between the 5th and 6th week of gestation. Affected babies have very few or absent T cells in blood, lymph nodes and spleen. Germinal centers are however normal and plasma cells are present in normal numbers. Infections start early with opportunistic organisms and yeast (*Candida Albicans*). They may develop pneumonia, especially due to the ubiquitous protozoa *Pneumocystis carinii*, and they can not terminate viral infections so that measles, chicken pox, vaccinia and herpes can all be rapidly fatal. Associated findings in this syndrome involve other congenital anomalies of the heart and face. These patients have no parathyroid glands because that gland arises in concert with the thymus embryologically therefore hypocalcemia and tetany are prominent clinical manifestations. Transplantation of fetal thymus gland has proven effective therapy in these individuals.

Severe Combined Immunodeficiency (SCID) (Alymphocytosis, Hereditary Thymic Dysplasia, Swiss Type agammaglobulinemia). This is always genetically induced. It may be inherited by X-linked recessive or autosomal recessive inheritance. Male to female ratio of affected infants is 3:1. Fifty percent of the patients with the autosomal recessive form of the disease lack the enzyme adenosine deaminase (ADA) or purine nucleoside phosphorylase (PNP). Either of the defects inherited as autosomal recessive traits can cause immunodeficiency. The metabolic consequences of ADA or PNP deficiency are such that ATP and deoxy-ATP accumulate in tissues. All patients with SCID lack T cells and have profound lymphopenia, most greater than 90%, and also have no B cells. They are thus agammaglobulinemic and have no delayed hypersensitivity and cannot reject grafts. An embryonal thymus gland lacking lymphoid elements is diagnostic of the disease. These patients are extremely susceptible to opportunistic infections. The disease is ordinarily fatal, though transplants of marrow from sibling donors may ameliorate the defects to a large extent.

Other congenital immune deficiencies have been shown due to defects in phagocyte cell function rather than to defects in T and B cell function. There are approximately 15 different inherited abnormalities of phagocytic function but four account for the majority of syndromes. These are chronic granulomatous disease of childhood (CGD), the Chediak Higashii Syndrome (CHS), the hyperimmunoglobulin E recurrent infection (Job's) syndrome (HIE) and myeloperoxidase deficiency (MPO deficiency). Although the cause of

their defective phagocyte function are all different, they share certain common clinical features, and they are predisposed to certain infections. Patients with CGD who have abnormal phagocyte oxygen metabolism most commonly become infected with gram positive organisms, both bacterial and fungal (staph, E coli, salmonella, aspergillus and nocardia). Patients with HIE syndrome have a predisposition to cutaneous infection with staph and candida and H influenza is cultured from respiratory tract and ear infection. Patients with CHS show no predilection for a specific organism but appear susceptible to all pathogens. Patients with MPO deficiency do not appear to have any problem handling bacterial infections but have difficulty with candida. The usual stand-by therapy for persons with these disorders includes appropriate and judicious use of antibiotics and in rare cases bone marrow transplantation to restore phagocyte cell function.

From observations on patients with immunodeficiency disorders much can be learned about normal resistance to infection. Specific antibody is important in protection against the pyrogens because complement is fixed by antibody and this results in opsonization and phagocytosis or bacteriolysis. Staphylococci, streptococci, pneumococci, hemophilus influenza, meningococci are principally disposed of by these two mechanisms of bacterial killing; intracellularly in phagocytes or by the bacteriocidal mechanisms. Furthermore specific antibody is important in protection against viruses which spread by viremia; e.g. measles, polio, rubella, hepatitis. Also mucosal surface antibody (IgA) prevents bacterial and viral adherence; e.g. gonococci, influenza virus. Specific antibody also neutralizes the toxins in tetanus, diphtheria and botulism.

Lymphocytes and macrophages are important in terminating intracellular parasitism such as occurs in TB, leprosy, brucellosis, tularemia, histoplasmosis, and all viruses. In some infections, both humoral and cellular immunity collaborate to prevent infection; e.g. syphilis, salmonella, shigella, monilia and listeriosis. Sometimes, in fact, the host immune response may cause the diseases like that which is seen in pertussis, pneumocystic pneumonia and ricketsial infection. Immune complexes may form with virus or bacterial products and cause injury as in bacterial endocarditis, malaria, syphilis and post-streptococcal glomerulonephritis.

The lesson to be learned from the study and elucidation of congenital immune deficiency diseases has been carried over into those of acquired origins. For example, widespread use of cancer chemotherapy agents had provided the clinician with a whole spectrum of clinical infections which occur as a consequence of suppression of the normal immune response. The clinician also faces the difficult task of overcoming such infections in patients whose immune response is markedly impaired. There is no greater clinical challenge than to get a patient over a temporary acquired immune deficiency such as that caused by the severe neutropenia and lymphopenia accompanying certain cancer chemotherapy regimens. Often by identifying the organism which is the offending agent one will have a clue to which component of the immune response is most markedly affected.

Similar knowledge is helping to describe and elucidate the current nationwide epidemic of AIDS. The occurrence of Kaposi's sarcoma (KS), of Pneumocystis carinii pneumonia or of other opportunistic infections (OI) in young previously healthy men was first documented in mid-1981. Between June 1, 1981 and June 7, 1983, 1,667 cases of these devastating illnesses were

reported from 35 states and 16 countries to the Centers for Disease Control (CDC). Because of the profound disturbances of cellular immune function underlying these illnesses they were grouped under the term of the acquired immune deficiency syndrome (AIDS). Although first recognized in homosexual men and intravenous drug users, the passage of time has revealed that other, unanticipated groups have increased risk. These include Haitians in the United States and Haiti, persons with hemophilia receiving factor VIII concentrate transfusion, prison inmates, infants in highrisk households and female sexual partners of men with acquired immune deficiency syndrome. Although AIDS conceivably occurred sporadically around the world in the past, it is certainly a new epidemic phenomenon in the United States, and, especially in New York and California. Cancer registries, autopsy files and data on pentamidine use in CDC do not indicate cases before 1979.

Patients with acquired immune deficiency syndrome, with or without Kaposi's sarcoma, experience multiple and often overwhelming infections with opportunistic organisms. It has been recognized that a higher incidence of less-conspicuous illnesses and laboratory abnormalities in high risk groups seem to be related to the acquired immune deficiency syndrome. These include prolonged unexplained lymphadenopathy with or without fevers and night-sweats, thrombocytopenic purpura, and non-Hodgkins lymphoma. The "lymphadenopathy syndrome" has apparently increased in incidence and may be premonitory of further progression of the AIDS epidemic.

Current evidence indicates that intimate sexual contact or blood transfer are the principal modes for AIDS spread. The presumed causal agent(s) has remained elusive during the 2 years since the first clinical reports. Persistence of the immune deficiency is a major feature of this illness and is compatible with a persisting causative agent.

The immune system changes that underlie AIDS lead to malfunction in host defense against infections and neoplasia. The immune deficiency is largely within the T-lymphocyte population. The B-cell numbers are normal. Serum immunoglobulin A and G levels are usually increased along with levels of circulating immune complexes in patients with opportunistic infections. Appreciable antibody titers to cytomegalovirus, hepatitis B, and sperm antigens have been noted. Antibody responses to primary immunization may be reduced. Bacterial infections, however, are infrequent.

A T-cell sub-population (the T helper group, which normally comprises 60% of the total T cells) characteristically is markedly reduced. In contrast another T cell subpopulation (the T suppressor/cytotoxic group) may be unchanged or increased. Furthermore, proliferative and cytotoxic and other T cell functions are reduced. The overall patterns of immune changes in AIDS are different from most other forms of immune deficiency. The study of AIDS like other aquired immune deficiency disorders serves to highlight the importance of immune mechanisms in host defense.

Asthma and Allergic Disease Centers

Taking advantage of the network of Asthma and Allergic Disease Centers the Allergy and Clinical Immunology Branch has established a series of semi-formal working groups, each comprised of those individual Center

Directors whose research activities are especially pertinent to that special problem. Additional selected members are chosen from the scientific community at large and a special emphasis is placed upon those individuals who are willing to work on a specific problem. During the past 18 months we have established a series of working groups and we will discuss the activities briefly. Additional information on any specific group can be obtained from the ACIB.

Occupational Allergic Disease Working Group (OADWG)

As a consequence of the February 1982 Symposium on OILD (published in Journal of Allergy and Clinical Immunology - July 1982), it was determined that recognition and prevention of occupational immunologic lung disease would depend upon team work. Individual problems are often so complex and difficult to define that a single scientific discipline does not have sufficient breadth to diagnosis, prevent and treat. In order to foster a collaborative role among occupational physicians, epidemiologists, allergists, clinical immunologists, pulmonary physicians and scientists with special expertise in chemistry and air sampling, we have convened 3 meetings of the OADWG and included diverse parties representing government at the local and state level as well as individuals from industry, academia and others.

Publicity for the activities of this group is being fostered by publishing a list of activities in the Journal of Occupational Medicine. In addition, Dr. Goldstein has met with representatives from labor as well as other individuals in order to develop a working agenda. To date, we have developed a proposed series of small studies. For example we currently intend to develop standard skin test reagents as an aid to the diagnosis of certain immunologically induced disorders. Materials under consideration include TMA, TDI, nickel and platinum. To date individual researchers must use whatever material is available to them in their locale, since no national standard is available. Specific studies aimed at testing the consequences of prevention are intended in the near term. In collaboration with the newly organized Foundation for Occupational Research, NIAID is also working to develop a national agenda to aid in cooperative research efforts in these disorders. It is hoped that knowledge gained by these studies in lung disease can be quickly applied to those of skin and eye.

As an offspring of the OADWG, we are organizing a workshop on Laboratory Animal Allergy, which is a significant occupational disease in the United States affecting Veterinarians, lab workers and other individuals. This workshop is scheduled to occur in Spring 1984 and designed to provide a forum for discussion of the scientific and policy issues in this area.

Drug Allergy Working Group (DAWG)

The importance of drug allergy as a significant cause of mortality and morbidity in the United States was emphasized in 1979 at the time of publication of the NIAID Task Force on Asthma and other Allergic Diseases. Subsequently it has become apparent that a variety of specific problems could be attacked in terms of finding useful diagnostic tools in order to detect individuals at risk for allergic reactions to drugs. In an effort to promote research in that specific area the ACIB convened the DAWG and presented an update of activities from a similar group already working in

Europe. Although NIAID already supports research efforts aimed at elucidation of penicillin allergy and a variety of other specific research grants, no central coordinating facility has yet become available.

As a consequence of this current initiative we are about to undertake a cooperative study among AADC's concerned with identifying individuals at risk to develop chymopapain reactions. Within the United States, and especially on the west coast, injection therapy with chymopapain for disc disease has begun during the past year. There have been few but definable number of severe reactions and even death in 1 or 2 rare instances. No organized approach to identifying individuals at risk has yet been undertaken in a collaborative manner in the U.S. This study should get underway in the Fall of 1983.

An additional effort emanating from ACIB includes an all day Symposium entitled Drug Allergy: Diagnosis, Prevention and Treatment which will be held in Washington on December 1, 1983. Proceedings of that Symposium will be published in the Journal of Allergy and Clinical Immunology. The purpose will be to provide the medical community at large with a comprehensive program of already available information which if applied clinically would hopefully reduce the numbers of persons experiencing allergic reactions to drugs.

Future efforts of the DAWG will be to develop and take advantage of the network of AADC's to promote targeted research in specific problems relevant to drug allergic reactions.

Food Allergy Working Group (FAWG)

As noted in FY 1982 the ACIB was in the process of making available a series of food extracts for both skin testing and oral challenge testing. These include milk, peanut, whole egg, wheat, soy bean, shrimp, rice, chocolate and corn. These materials have now been made available under an Investigational New Drug protocol which is shared between NIAID and the Mayo Clinic Research Reference Laboratory.

During the past year, we have convened on two occasions the FAWG which is comprised of those investigators, mostly funded from NIAID, who are already performing specific studies in a variety of aspects of food allergy research. The purpose of FAWG is to bring together these groups that they may find mutual areas for collaborative research to occur. One of the initial projects under consideration is the development of a common informational protocol that would permit central accumulation of data among patients in whom test materials have been utilized. In that manner we hope to obtain data on these individuals, a body of data that has never to our knowledge been acquired previously. It should be mentioned also that a variety of research projects are currently funded in food allergy, including measures of effects on behavior as well as efforts at purification of specific materials.

Aspergillosis Working Group

The clinical problem of aspergillosis in the United States appears to be on the increase, especially in two prominent patient populations. First there appears to be a very marked number of individuals with cystic fibrosis

who suffer from Allergic Bronchopulmonary Aspergillosis (APBA). In addition, there is evidence to suggest that individuals with chronic steroid-dependent asthma may have undiagnosed cases of ABPA. Secondly superinfection with fungus ball (aspergilloma) is a prominent cause of moribidity and death in patients with chronic lung disease. In both instances there are no "standard" diagnostic reagents available for either research or clinical use. Because of the importance of this problem, the ACIB convened a small group of investigators in hopes that an organized collaborative approach to this problem might result in progress that could not otherwise occur. At the present time a standard reagent is being prepared which will be available for specific research use in performing the appropriate epidemiologic studies. Also standard measures of anti-aspergillus antibody will be made available through one of our AACD laboratories. Plans for the next year include extension and use of these materials in selected clinical populations.

Centers for Interdisciplinary Research on Immunologic Diseases

Among the special projects emanating from our CIRID program was the development at UCLA of the Asthma Care Training program for self-management of childhood asthma. In the past decade there has been a renaissance of scientific interest and research in health education and self care, particularly as they apply to chronic disabling disease. A number of programs evolved, some with research funding and some as grass roots efforts. In June 1981 our CIRID at UCLA in conjunction with NIAID and AAFA sponsored a Conference on Self-Management of Asthma where many diverse programs were presented and discussed. As a consequence of that program it became apparent that we lacked sufficient critical information to evaluate these diverse activities. Therefore in conjunction with NHLBI, NIAID developed a program to critically evaluate this field in order to provide guidance to those individuals to develop programs of their own or wishing to utilize currently available programs. In June 1983 NIAID-NHLBI convened a Workshop on Self Management of Childhood Asthma. The Proceedings will be published as a supplement to the Journal of Allergy and Clinical Immunology in November 1983. As a consequence of the evaluation it became evident that data now exists to support the efficiency of establishing such programs in selected communities. The CIRID program at UCLA (ACT) was a notable example of a well developed program where application in the community has shown beneficial results. It is the current intention of NIAID to work along with the UCLA CIRID in developing sufficient teaching materials to distribute to CIRID and AACD programs throughout the United States. A Spanish language version of the ACT program is under development for use in selected urban areas.

The problem of Asthma is considered an important one for this Institute. In addition to supporting the educational program and specific research projects in both environmental and allergic asthma, NIAID in conjunction with the American College of Chest Physicians and the Academy of Allergy and Immunology, is planning a jointly sponsored 2 day educational program in April 1983 at which time new developments in the diagnosis, treatment and prevention of asthma will be reviewed by individual investigators from both the allergy and pulmonary community. The results of that State of the Art Symposium will be published in a special issue of CHEST that will be widely distributed to family practitioners, general

practitioners and internists who are primarily responsible for treating the many asthmatic patients in this country.

Lastly NIAID intends to organize a trial of immunotherapy in asthma during FY1984. Whether or not immunotherapy is efficacious in childhood and adult asthma, has been a subject of much controversy. Because purified and well characterized allergenic extracts are now available, a study of efficacy can be accomplished among several cooperating Centers in hopes of answering some of these controversies.

Resource and Reference Lab

After the establishment of the Reference Lab at the Mayo Clinic in FY 82 we have been able to make available a series of new reagents for allergic and immunologic research. The initial three international reference preparations short ragweed, mite, and Timothy grass prepared in collaboration with the International Union of Immunologic Societies Subcommittee on Allergen Standardization of the World Health Organization, are ready for distribution to investigators pending final acceptance by WHO as International Reference Standards. Five additional extracts are in the final stages of preparation. These include alternaria, cat, dog, bermuda grass and birch. Previously mentioned food extracts have been made available to collaborators in the food allergy working group. A series of other standards including C3, and ELIZA for IgE and Ragweed Antigens E and K have also become available.

Genetics and Transplantation Biology Branch

I. Administrative Summary

A. Scope

This Branch supports and manages research on the immunologic and genetic factors that determine the acceptance or rejection of grafts.

Studies supported by the Branch are focused on antibody and cellular mechanisms that mediate graft rejection. Included in these efforts are studies on the subsets of T cells that are recruited by the recipient in response to allograft transplants, as well as the role of lymphokines e.g. IL-1, IL-2, in mediating graft rejection. Other research efforts supported by this Branch include: (1) elucidation of the structure, organization and control of the expression of genes which regulate immunologic activity; (2) studies of strategies designed to minimize the likelihood of rejection by assuring the best possible antigenic match between donor and recipient; (3) development of immunosuppressive regimens whose objective is to reduce, or eliminate, the capacity of the recipient to reject a graft; (4) development of protocols for rendering the graft unable to provoke an immunologic response in the recipient.

Animal models studies are supported in order to explore the genetic regulation of the host immune response, and, in a more practical sense, to test methodologies whose ultimate application is to the human patient.

B. Organization

Research activities supported within this Branch are grouped into the general areas of Transplantation Biology and Immunogenetics. Program Projects for Transplantation Immunology are also supported. This Branch further supports a contract activity which provides reference reagents to facilitate ongoing research.

1. Transplantation Biology

This program is concerned with the cell biology of lymphocytes, macrophages and monocytes regarding their function in recipient - graft interactions. Research supported within this program includes studies on the origin, maturation and function of immunologically active cell populations in response to allogeneic stimulation as a result of organ transplants. Relevant studies are conducted on the role lymphokines play in the activation and recruitment of cells that mediate graft rejection. Studies of the molecular genetics of Class I and II major histocompatibility antigens (MHC) and their role in allogeneic responses in terms of cell-cell surface interactions are also supported by this program.

2. Immunogenetics

Research in this program area focuses on identification of cell surface antigens of the major histocompatibility complex, the cloning of genes that regulate the expression of these antigens, and development of monoclonal antibodies specific for these antigens that can be used to identify lymphocytes that function in allogeneic responses. Particular emphasis is placed on the structure

of Class I and II major histocompatibility antigens (MHC), and to clone those genes that regulate the expression of these antigens on cell surfaces, and to devise in vitro and in vivo assays for monitoring host responses to these antigens. Animal studies are supported, where inbred, congenic and recombinant strains of well defined animal populations are used to assay allogeneic responses, and to devise treatment modalities to alter or suppress these responses, before application to man.

3. Program Projects in Transplantation Immunology

The practice of transplantation has evolved to the point that the technical aspects of the surgical procedures are no longer limiting. The major remaining hazards are associated with the rejection process and with the immunosuppressive therapy employed to control or prevent rejection. Thus, this program area is designed to investigate the multifaceted aspects of allogeneic stimulation and response induced by tissue transplantation in the human patient. Currently, three program projects are supported that intergrate the expertise of cell biologists, cellular immunologists, clinical immunologists, geneticists, transplant surgeons and each is directed by an acknowledged leader in the field. Their combined efforts are designed to probe and elucidate the mechanisms controlling and mediating alloantigen stimulation that ultimately lead to graft rejection. These projects employ state of the art methodologies in molecular genetics, monoclonal antibody production, polymorphic cell surface antigen identification, in order to identify and assay cellular function in graft rejection.

These Program Projects Emphasis represent a major financial and philosophic commitment by the IAIDP and NIAID to elucidate the cellular mechanisms involved in graft rejection.

4. Reagents and Resource Contracts

The contract mechanism has been employed to assist current research efforts by providing useful and unique reagents to investigators.

The U.S. population consists of many ethnic groups, which present the opportunity to define unique HLA antigens, that are not well defined, particularly within the Black population. Two contracts were previously awarded to define and acquire alloantisera specific for HLA antigens within this population. Efforts to improve the definition of histocompatibility antigens in the Black population has increased with the funding of a third contract. A unique aspect of this most recently funded contract is the ability of the contractor to detect, define and acquire anti-idiotype antibodies as well as the standard histocompatibility reagents.

Over one thousand serum samples obtained from parous Black women have been screened to detect antibodies to poorly defined or previously undefined antigens of the major histocompatibility complex. Approximately fifteen percent (15%) were found to contain useful antibodies to HLA-A, B, C, or DR antigens. Many of the antibodies identified are specificities that have been in limited supply in the past. Of those serum samples identified as containing useful antibodies, fifteen percent (15%) are specific for antigens found more frequently in Blacks, or appear to identify previously undefined antigens. Routine exchanges of these reagents between contractors have increased the speed with which these specificities can be

fully defined and become integrated into routine clinical typing procedures. The acquisition of a larger array of reagents identifying these more poorly defined antigens, and the identification of a greater number of cells bearing these antigens have led to the realization that the BW35, BL5, BU and SV crossreactive group is more complex and extensive than previously believed. The same also seems to be true of the AW19 crossreactive group. Special attention will be focused on analyzing data from the screening of cells and sera recognizing components of these crossreactive groups to develop a better discrimination of the various subunits of these complex antigen. Thus, the contracts awarded for assessing the Black HLA antigens have provided a better understanding of HLA antigens within the human population of the U.S. This information plus the acquisition of the specific alloantisera will further benefit those patients in need of organ transplants.

The facilitation of antigenic cross-match between a potential donor and recipient is being studied through a contract awarded to develop a rapid cellular cross-match for use in cadaveric organ transplantation. This contract is designed to reduce the time required for cellular cross-matching from 3-5 days to 12 hours or less. Preliminary results have identified several "activation" antigens expressed early in the lymphocyte activation process. Therefore, these antigens are candidates for indices of early responses to alloantigens which can be used to develop a definitive assay. Additional studies to define the most efficient test system have also been completed. The use of a B-cell enriched stimulator population produces an earlier, more vigorous response than an unfractionated lymphocyte population. The contractor will be encouraged to pursue the investigation of other potentially useful test systems, including the use of primed lymphocytes as the responder population. Thus, the studies carried out to date are very promising and indicate that the desired quick response format can be developed which could easily be incorporated into the work flow of existing histocompatibility laboratories.

In addition, a series of individual tasks orders are being issued under the Master Agreement entitled "Develop and Supply Serologic Reagents for the Investigation of Polymorphic Human Cell Surface Antigens." The intent of these task orders is to acquire for the NIAID Serum Bank specific histocompatibility reagents which are currently in limited supply in its inventory. The reagents will be supplied to qualified investigators to support their research in transplantation immunology and human immunogenetics.

C. Awards and Support Levels

Relevant activities in transplantation immunology and immunogenetics are supported through various mechanisms including contracts, individual post-doctoral fellowships, institutional pre-and post-doctoral training grants, career and career development awards, as well as investigator initiated research grants.

The following shows the distribution of support for the activities of the Branch during FY 1983.

Genetics and Transplantation Biology Branch

FY 1983 Awards

<u>Award Mechanism</u>	<u>Number</u>	<u>Amount *</u>
Research Grants	104	\$14,082,699
Career and Career Development Awards	3	\$ 114,843
Subtotal	107	\$14,197,542
 Fellowship Awards	12	\$ 252,765
Training Awards	0	0
Subtotal	12	\$ 252,765
Contracts	7	\$ 726,465
 Total	126	\$15,176,772

*Total costs, indirect costs estimated

The majority of these awards are concerned with either transplantation immunology or the molecular genetics of the major histocompatibility antigens. Approximately 33% of these awards were for competing new or renewal applications; the remainder represents commitments to support awards made in prior years.

Support for program projects in Transplantation Immunology are included in the above grant category. During FY 1983, three program project awards, at a cost of \$945,647 were made to support this activity.

II. Scientific Summary

Advances made in transplantation immunology have led to an increase in awareness in both the public and political arenas. This awareness was brought about by the increased survival of transplanted recipients e.g. average of 84% at the end of three years for renal transplant patients from cadaveric and related donors. Thus, the House Subcommittee on Investigations and Oversight on Science and Technology held three days of hearings on April 13, 14 and 27, 1983, the subject of which was Human Organ Transplants. Transplant surgeons and scientists (supported by NIAID) plus other witnesses gave testimony on the advances in the field of transplantation immunology. These hearings were followed up by a meeting convened by the Surgeon General, PHS, DHHS on June 7-9, 1983. The subject of this meeting was to outline and draft resolutions to the problems of the shortage of human organ donors! This meeting was attended by both NIAID staff and NIAID supported scientists and surgeons. These hearings and meetings emphasize the rapid advances being made in the science of transplantation immunology to elucidate the immune responses to alloantigens i.e. organ transplants thereby enhancing graft survival. NIAID through contracts, investigator initiated

research grants and program projects supports the foundation upon which most of the advances in human transplantation is made possible.

Transplantation Immunology

Advancements in the field of transplantation immunology occur largely as a result of elucidating the mechanisms of allogeneic responses, cell cloning and development of immunosuppressive modalities. An objective to the studies on allogeneic stimulation/response is to define the cell populations, and subsequent immune response, that result in acute allograft rejection. Progress made during the past year revealed that the mechanism responsible for Bone Marrow Transplant (BMT) rejection, differs from solid organ rejection. One study demonstrated that transfusion of pure platelets into appropriate recipients sensitizes these individuals to donor BMT, with subsequent rejection of hemopoietic stem cells. The interesting results of BMT rejection, which contrast markedly with solid organ rejection are: (1) in vitro cellular cytotoxicity assays were negative; (2) antibody specific for transplantation antigens was not produced; (3) skin graft rejection was not accelerated. These data suggest that the immunobiology of BMT acceptance or rejection differs from solid organ transplants.

Other studies have identified discrete populations of T-cells whose function during grafts undergoing acute allograft rejection differ from each other. Addition of these T cells subsets to separate B recipients (T cell depleted animals) whose renal grafts are not rejected, causes either no rejection in one group or the induction of renal allograft rejection. Appropriate cell cloning and immunosuppressive techniques will lead to further definitions of cell function in order to enhance graft survival.

Cell cloning has become a very important tool in transplantation immunology. Progress during the past year identified functionally distinctive cells which were cloned to produce mediators of lymphocyte function, suppressive agents, and biologically distinct populations in sufficient numbers for cell surface antigen studies and monoclonal antibody production. One study revealed that cultured Sertoli cells produced an immunosuppressive substance that inhibits cellular responses to alloantigens. Much of the progress in these areas was in the production of large cell populations, via cell cloning, which allowed cell surface antigens to be identified and isolated through state of the art immunochemical techniques. This resulted in the production of monoclonal antibodies, specific for the MHC cell surface antigens, allowing for cytometric analysis of lymphocyte populations. Other studies on human non-MHC antigens resulted in monoclonal antibody synthesis that identify and, are specific for leucocytes and endothelial cells surface antigens that are "passengers" on organ transplants, which often play a role in graft rejection. Collectively, these results enhance our understanding of the role cell-surface antigens and cell function play in graft survival.

Molecular Genetics

The study of transplantation has been greatly facilitated by the advances made in gene cloning. Progress made during the past year focused on the Class I (H-2K, D, L of mouse, HLA-A, B, C of man) and Class II (H-2I of mouse, HLA-DR of man) MHC antigens. Genes were cloned that not only control immune responsiveness, but also those that regulate expression of MHC antigens on cell surfaces. Interesting

results demonstrated that a gene encoding B_2 -microglobulin is responsible for transporting MHC antigens to the cell surface². These results were based on the isolation of mutant cell lines that lack this particular gene, and thus lack cell surface MHC antigens. Insertion of the B_2 -microglobulin gene into these cell lines, results in the expression of MHC antigens. Further, the insertion of this gene into mutant cell lines, induces some of these cells to synthesize antibody. Thus, an interesting question posed by these studies focuses on the expression of MHC antigens on all cells, yet antibody is restricted to a certain cell populations, both functions seemingly regulated by the B_2 -microglobulin gene.²

Further studies identified a translocation of the c-myc gene from chromosome 15 to the heavy chain region of chromosome 12, in mouse myelomas. This insertion of the c-myc gene into the coding region (exon) of the switching region of chromosome 12 suggests a possible alteration in the expression, synthesis (function) of myeloma cells. In fact, three exons were identified, that transcribe at least six different c-myc RNA transcripts, affecting cellular transformation.

Polymorphic Cell Surface Antigens

Cell surface antigens play the major role in alloantigen stimulation in transplanted recipients. As stated above, the major thrusts in this area during the past year were on cell cloning, function, surface expression and the genes that regulate these activities. However, another allogeneic response occurs between females and males in response to the male H-Y cell surface epitope. Results during the past year demonstrated that female C3H mice reject male skin grafts only after splenectomy or thymectomy. Further, female C57BL/6 mice become non-responsive to H-Y antigen as a result of multiparity. Analysis of T cell populations with Lyt monoclonal antibody demonstrated profound population changes in T lymphocytes in female mice as a result of splenectomy or multiparity. Results of these studies indicate that surgery or hormonal alterations prior to transplantation can affect graft survival.

It is evident that research into the mechanisms that play major roles in allograft rejection and/or survival are being identified and dissected by the research efforts supported by this Branch. It is anticipated that due to the high quality of research, further progress will be made in enhancing graft survival in the transplanted recipient.

IMMUNOBIOLOGY AND IMMUNOCHEMISTRY BRANCH

I. Administrative Summary

A. Scope

This Branch is concerned with the biology and chemistry of the immune system and its products. The fundamental studies which it supports on the structure and function of the immune system are directed toward acquiring a complete understanding of immune response mechanisms at their basic cellular and molecular levels as they function in health and disease.

Activities in this broadly based program area cross traditional disciplinary lines of biology and chemistry and encompass anatomic, physiologic, pharmacologic, and microbial biology and organic, physical, and biological chemistry. Its scope includes studies of the origin, maturation, localization, and function of immunologically active cell populations, and the mechanisms involved in the induction, modulation, regulation, and expression of immune reactivity, as well as physicochemical studies of antigens and their homologous antibodies and the mechanisms and kinetics of antigen-antibody reactions.

B. Organization

Research activities supported within this Branch are grouped in the general programs for Immunobiology and Immunochemistry and in a special category limited to Program Projects in Lymphocyte Biology. This Branch also supports a contract activity which provides research reagents and materials to facilitate ongoing research.

1. Immunobiology

This program is concerned with the processes leading to immunocyte differentiation, proliferation, and production of biologically active substances which mediate immune reactions. Research supported within this program includes studies on the origin, maturation, and localization of immunologically active cell populations, the interactions of lymphocyte subpopulations and their interrelationships with macrophages and other leukocytes, the cellular phenomena of antigen processing, immunologic tolerance and enhancement, and the mechanisms involved in the induction, modulation, and regulation of immune responses. The cellular mechanisms involved in the induction, maintenance, expression and pathophysiology of delayed type hypersensitive immune reactivity are also included in this category. Also relevant are studies of the lymphokines and other lymphocyte substances which activate macrophages and have pharmacologic effects on lymphocyte and other leukocytic cell functions such as chemotaxis, phagocytosis, blastogenesis, cytotoxicity, and microbial resistance.

2. Immunochemistry

Research grouped in this program category focuses on the defined molecular components of the immune system with particular emphasis on elucidating the molecular basis of the regulation of the immune response utilizing chemical and physicochemical techniques for study of immunologic reactants and reactions. A major aim of studies supported by this program is to elucidate the structure of antibodies with emphasis on their antigen binding, specificity, and other

biological functions. A second objective of this program is to elucidate the critical aspects of antigen structure that determine immunogenicity of the antigen.

3. Program Projects in Lymphocyte Biology

This activity currently supports seven program projects, each of which is a multidisciplinary research effort integrating the technical expertise of cell biologists, geneticists, cellular immunologists and immunochemists and is directed by an acknowledged leader in the field. Their combined studies are designed to expand knowledge of the morphologic and functional heterogeneity of lymphocyte populations and to develop the capability for identification and selection of lymphocyte subpopulations with specific immune reactivity or antigenic composition, for hybridization of such populations and for selective production of specific lymphocyte products. The objective of this Program is to acquire as complete a knowledge as possible of the life history of immunocompetent cells and of the physiologic and external factors that determine their fate and function *in vivo* and *in vitro*.

This program project activity represents a major financial and philosophic commitment by the IAIDP and the NIAID to definition of the biology of the lymphocyte and the immune system in general. Support for carefully organized efforts by closely interacting groups, led by well established and highly productive senior scientists, through the program project mechanism has proven to be successful and effective, both scientifically and administratively.

This program was initiated approximately eleven years ago with the award of four program project grants, each directed toward the goal of understanding the biology of the lymphocyte and having different but complementary approaches. Since then, three of the original projects have received renewal support and two new program projects were established, one each in FY 1978 and FY 1979.

The productivity of these select groups has been exceptional and their varied contributions have been, and continue to be, the stimulus for the intense current efforts in this field. In fact, many of the initiatives identified for concentrated development during this decade in the recent report of the NIAID sponsored Immunology Study Group have been, and are, within the scope of the Lymphocyte Biology program project activity. In this context, during FY 1981 the Institute considered the future of this activity and decided, with the concurrence of the National Advisory Allergy and Infectious Diseases Council, to continue it in its current form. Accordingly, a Request for Applications was published in March, 1981 announcing a competition for awards in FY 1982. Applications were received from the two active program projects scheduled for competitive renewal and from an additional investigative group. These applications were reviewed for scientific merit in October, 1981 and by the Advisory Council in January, 1982; as a result, renewal of support for the two program projects was awarded in May, 1982.

An identical Request for Applications was published in November, 1981 announcing a competition for awards in FY 1983. Applications were received from the two active program projects scheduled for competitive renewal and from four other investigative groups. These applications were reviewed for scientific merit in June, 1982 and by the Advisory Council at its September, 1982 meeting. Renewal of support for the two active program projects was awarded in May, 1983 and two new program projects were established.

The following listing identifies the currently active program projects and shows the term of their award.

Albert Einstein College of Medicine (1972-1987)
Rockefeller University (1973-1985)
University of Texas, Dallas (1974-1984)
Harvard University (1978-1988)
Jewish Hospital of St. Louis/Washington University (1979-1987)
Scripps Clinic and Research Foundation (1983-1986)
Stanford University (1983-1986)

Consistent with Institute policy, a Request for Application was published in August, 1982 announcing a competition for award in FY 1984. A renewal application was received from the Dallas group; it was reviewed for scientific merit in June, 1983, and is scheduled for consideration by the Advisory Council at its September, 1983, meeting.

4. Reagents and Resources Contracts

The contract mechanism has been employed to assist current research efforts by providing useful and unique reagents and materials to investigators.

On recognition of the important functional roles that lymphocyte cell surface molecules exert on the immune system, contract awards have been made for the acquisition and distribution of antisera with specificities directed against various mouse cell surface antigens to facilitate research in this area. A supply of antisera against many of the H-2 gene products of the murine major histocompatibility complex (MHC) has been made available for distribution and antisera specific for antigens of the I region of the H-2 complex have been prepared and are available for distribution. Similarly, the acquisition and distribution of Ly antisera and the preparation of antisera against newly recognized Ly antigens and other immunologically relevant cell surface molecules are being supported by a contract mechanism. Interest in these reagents remains high; approximately 1,200 ml of these antisera were distributed in 128 shipments to 95 investigators during the current year.

The contract awarded for the acquisition, characterization, storage, and distribution of hybridoma cell lines also is intended to support and facilitate investigator initiated research studies of the immune system. During the first three years of operation, 160 hybridoma cell lines have been acquired; 94 are now available for distribution and 66 are being characterized and propagated for subsequent storage and distribution. The specificities of the antibody products of these cell lines cover a broad range of research interests including among others, antimicrobial, anit-H-2, anti-Ly, and anit-human lymphocyte cell surface antigens. It is estimated that during the current year, approximately 1500 cell lines will be distributed to approximately 460 investigators.

Support is being provided for continuation of a trans-NIH contract project which serves as a single reference resource and data base for amino acid sequences of immunoglobulins, H-2, Ia, and related human and murine MHC products. Three books containing detailed sequence data, have resulted over eight years during which this project has been active. Current efforts include the continued collection, tabulation, and analysis of amino acid sequences as well as expansion to the nucleic acid sequences of the genes controlling the synthesis of these molecules.

C. Awards and Support Levels

Relevant activities in immunobiology and immunochemistry are supported through various mechanisms including contracts, individual post-doctoral fellowships, institutional pre- and post-doctoral training grants, career and career development awards, as well as investigator initiated research grants.

The following shows the distribution of support by award mechanism for the activities of the Branch during FY 1983.

Immunobiology and Immunochemistry Branch FY 1983 Awards

<u>Award Mechanism</u>	<u>Number</u>	<u>Amount*</u>
Research Grants	220	\$ 30,187,731
Career and Career Development Awards	13	\$ 522,052
Subtotal	233	\$ 30,709,783
Fellowship Awards	19	\$ 311,186
Training Awards	8	\$ 1,030,864
Subtotal	27	\$ 1,342,050
Contracts	4	\$ 535,021
Total	264	\$ 32,586,854

* Total costs, indirect costs estimated

The distribution of these awards by discipline is approximately 4/5 for immunobiology and 1/5 for immunochemistry. Approximately 40% of these awards were for competing new or renewal applications; the remainder represents commitments to support awards made in prior years.

Support for the Program Projects in Lymphocyte Biology is included in the research grant category. During FY 1983, seven program project awards, at a total cost of \$4,542,409 were made to support this activity.

II. SCIENTIFIC SUMMARY

Fundamental studies of the immune system have been in a transitional phase, shifting from descriptive to analytical approaches, as a result of the application of recently developed technology. The experimental immunologist now has the capability to visualize and isolate specific cellular components of the immune system for precise studies of the molecular mechanisms responsible for their functional activity. With appropriate monoclonal antibodies prepared by hybridoma technology, these cells can be identified and sampled using the fluorescence activated cell sorter. By suitable culture techniques, the selected cells can be cloned and propagated in large volumes to provide a sufficient supply of sub-cellular components for molecular characterization. Isolated fractions can be further defined by analytical techniques, providing detailed structural information

and permitting definition of the nucleotide sequences controlling the synthesis of important cellular constituents. Using this information, relevant molecules can be synthesized in sufficient quantity and purity by recombinant DNA technology to permit detailed studies of their functional role in the immune system. With such capabilities, contemporary immunologists are exploring the molecular biology of the immune system with intensity to acquire a complete understanding of its normal and abnormal function.

A full understanding of the ontogeny of immunocompetent cells is required for selective manipulation of the immune system and for correcting imbalances and deficiencies which compromise host immune defenses. For this reason definition of the origin, differentiative pathways, and functional roles of the various subpopulations of lymphocytes continues to be a focus of considerable investigative effort. Although it is certain that one population of mature lymphocytes (T) is thymus dependent and another population (B) is thymus independent, their progenitors and their mechanisms of functional maturation are still not precisely identified. Studies of T and B cell lineage have been greatly facilitated by the recognition that certain lymphocyte surface molecules are characteristically expressed at specific stages of differentiation and maturation and by the availability of specific antisera to these marker antigens.

Emphasis currently is being placed on B cell ontogeny because it has been recognized that events controlling the expression of immunoglobulin genes occur very early in B cell development, providing the opportunity to identify the genetic mechanisms responsible for immunoglobulin synthesis and diversity, and that many of the clinically relevant abnormalities in antibody producing cells reflect defects which occur and become evident very early in B cell differentiation. Furthermore, monoclonal antibodies against phenotypic and functional markers that define a series of sequential steps in B cell differentiation have been developed and provide the means for separating and studying independently the characteristics and functions of cells at each developmental stage. Transformed cell lines representing many of the stages of B cell differentiation also are available and hybridoma technology has provided the opportunity to arrest and maintain normal B cells at varying developmental stages. It is likely that techniques for developing and maintaining clones of genetically unaltered B cells soon will be available.

Using such approaches, considerable information concerning the regulation of B cell differentiation has been obtained recently. It is now generally accepted that the developmental process leading from multipotential stem cells to pre-B cells to immature B lymphocytes bearing cell surface immunoglobulin M (IgM) are genetically programmed and are not antigenically driven. Nevertheless, the respective roles of self or exogenous antigens, mitogens, or T cell factors, in contrast to intrinsic genetic programs, in mediating succeeding steps in differentiation, particularly the expression of diverse immunoglobulin isotypes, are less well defined.

Analysis of the expression of isotype diversity has contributed importantly to developing current understanding of the process of B cell differentiation. Sequential changes in expression of isotypes in the cytoplasm or on the surface of B cells during ontogeny or after antigenic or mitogenic stimulation have served as the major phenotypic markers for discerning sequential steps in differentiation. In the recent past, the segment of mouse chromosome 12 which contains the family of genes encoding immunoglobulin heavy chains has been analyzed in considerable depth and detail. The resulting information concerning the organization and structure of immunoglobulin genes in their germline configuration and the changes in context and content of these genes that accompany B cell differentiation, coupled with the

biological understanding of the differentiation process, has provided the opportunity for gaining a precise understanding of the molecular mechanisms which mediate the process. Experimental data indicate that development of isotype diversity is an intraclonal process that begins with expression of IgM by an immature B cell and leads to a family of plasma cells and memory B cells which express the same immunoglobulin light chains in conjunction with each of the different heavy chain isotypes. Current genetic evidence suggests that isotype switching is accomplished by chromosomal translocation of immunoglobulin gene complexes during differentiation. Further definition of the genetic mechanisms involved in immunoglobulin synthesis and isotype switching may soon provide experimental and clinical immunologists the capability to genetically engineer the system as necessary to either enhance or abolish a specific immunoglobulin isotype.

Although the functional distinction of lymphocyte populations is convincingly documented, the purpose and mechanisms of their interactions with each other and with macrophages is still being examined. It is clear that B lymphocytes differentiate to become antibody-secreting plasma cells and that differentiated T lymphocyte subsets can exert helper or suppressor effects on B lymphocytes as well as effector functions in transplantation reactions and in cell-mediated immune reactions to various microbial agents. The regulatory and effector functions of murine T lymphocytes have been found to correlate well with the presence of the cell surface Ly antigen. It also is known that these functions are controlled and regulated by other cell surface antigens which are products of the genes in the MHC. The regulatory role of MHC products and the functional role of other cell surface antigens in the immune response have been defined most extensively in the mouse although systems analogous to the H-2 component of the murine MHC have been identified in other animals and in man.

Evidence obtained in several experimental systems has convincingly demonstrated that efficient physiological interactions among macrophages, T cells, and B cells require that these cells share membrane molecules encoded for by the MHC of the species. The genes controlling interactions between T and B lymphocytes are located in the I region of the mouse H-2 complex; T Lymphocytes will not exert effective helper functions for B cells when these cells differ at the relevant I region locus. Genetic restrictions also are imposed by products of the I region on interactions between macrophages and immune T cells. Naive lymphocytes will respond to an antigen even if it is presented on a histoincompatible macrophage. However, the secondary immune response is genetically restricted; the immune T cell must be stimulated with antigen-bearing macrophages which are genetically identical to the macrophage that presented the antigen during the primary immunization.

The molecular basis for genetic restriction of the immune system is the subject of intense interest now that the technology of gene cloning has been applied to the MHC. The complete nucleotide sequence of several MHC genes has been determined and, within the immediate future, it is expected that the complete molecular structure of representatives of the major classes of MHC genes and the protein products encoded by these genes will be available. The structural definition of MHC gene products will provide the opportunity to clarify the molecular basis of the genetic restriction of immune responses, as well as of the cell-cell recognition process which can play a key role in transplantation, autoimmunity and in resistance to a variety of infectious and neoplastic diseases.

The existence and nature of the T cell receptor, a putative surface structure through which T cells recognize non-self antigens, still provide a challenging area for investigation. As long as the identity of the T cell receptor is undefined, a molecular understanding of the cellular interactions underlying self-recognition and activation of immunologically competent T cells is not possible. Considerable insight into the molecular and functional properties of the receptor has been obtained recently using monoclonal antibodies that recognize T cell surface proteins with predicted characteristics of the receptor. The reactive molecule has been detected only on the surface of mature, immunocompetent T cells and, as expected, has antigen specificity. Amino acid and nucleotide sequences are being obtained to detect and map the chromosomal position of the responsible genes for subsequent studies of the origin of diversity in T cell receptor specificity and to define the relationship between genes which code for the T cell receptor and those coding for immunoglobulins. It is anticipated that definition of the nature of the T cell receptor also will be useful in clarifying conflicting views on the mechanisms of antigen recognition by T cells and of genetic restrictions of immune responsiveness.

Future applications of gene cloning technology may also clarify understanding of the immunoregulatory functions of T lymphocytes, a topic of considerable current interest which has gained great impetus recently with the unexplained appearance of the human acquired immune deficiency syndrome (AIDS) in major urban areas throughout the U.S. Although the etiology is unknown, the susceptibility of AIDS patients to infection by opportunistic pathogens has clearly been associated with a loss of immune response capability and, most likely, a perturbation of immunoregulatory mechanisms.

For some time now, functional subsets of mouse T lymphocytes have been identified on the basis of their surface Ly antigens; helper T cells have been shown to be Ly1+ while suppressor cells are Ly2,3+. Human homologs, both structural and functional, of the murine Ly antigens also have been described; monoclonal antibodies against the T4 surface antigen detect the human helper T cell subpopulation while suppressor T cells react with an anti-T8 monoclonal antibody. AIDS patients characteristically exhibit a profoundly reversed ratio of T4 to T8 cells, consistent with their markedly suppressed immune responsiveness. These surface molecules, which obviously are associated with immune recognition and regulation, are products of differentiation genes which are outside of the MHC but are believed to be located on the same chromosome. The eventual cloning of these genes and the structural definition of their products will permit a clarification of their immunoregulatory mechanisms at a molecular level and may lead to a therapeutic approach for correcting immunoregulatory deficits such as those of AIDS patients.

Interest also is currently focused on soluble immunoregulatory products of T lymphocytes. It is clear that soluble helper and suppressor factors are released by their respective cells and that these factors are functional when added to antigen-stimulated cell cultures. The potential use of such soluble immunoregulatory factors in the intact host has served as a stimulus for study of the molecular nature and mechanisms of action of these factors. The possibility of achieving immunoregulation with cellular products is exciting because it avoids the recognized complications of cellular transfusions. For this reason, among others, efforts to produce soluble immunoregulatory factors in large volumes and in relatively pure preparations are increasing. Hybridoma technology is one possible approach which has met with some success in the murine system; a T suppressor hybridoma has been developed and its suppressor factor has been reported to possess functional and chemical properties identical to those of the parent suppressor T cell.

Another active area in immunoregulation concerns the unique antigenic determinants or idiotypes associated with the variable regions of antibodies, receptors, and antigen-binding factors. These idotypic markers constitute a target for the regulatory action of idotype-specific antibodies, T lymphocytes and other soluble factors. Significant progress has been made recently in elucidating aspects of the regulatory process which involves idotype-antiidotype interactions. Treatment of mice with antiidiotypic antibody has been demonstrated to abolish delayed-type hypersensitive reactivity by the action of suppressor T cells; certain antibody responses also have been found to be inhibited by treating mice with antiidiotypic antibody. The unique specificity of antiidiotypic antibodies adds to their potential for use practically as immunoregulatory agents. Hybridoma technology is now being employed to prepare monoclonal antiidiotypic antibodies in quantity for subsequent investigation of their immunoregulatory potential.

The related area concerning interleukins also is receiving considerable investigative attention. The interleukins are products of stimulated cells; lymphokines, also known as interleukin II, are produced by activated lymphocytes while monokines, interleukin I, are released by activated monocytes. These cell products exert multiple biological effects and regulate immunological and inflammatory host responses by serving as intercellular messengers which modulate cellular functions. Their biological activities are immunologically nonspecific and genetically unrestricted but have proven to be useful to experimental immunologists. Interleukin I has been shown to enhance B lymphocyte antibody production, promote lymphokine production and augment thymocyte proliferation. Interleukin II has been demonstrated to have a variety of biologic activities including, among others, non-specific helper function, T cell growth promoting ability, and thymocyte maturation capability. The interleukins represent a valuable tool for study of inflammation and, as individual molecules are isolated from this heterogeneous group, efforts to prepare monoclonal antibodies against them for use as anti-inflammatory agents are anticipated.

The most extensive study of the interleukins is centered on the T cell growth promoting activity of interleukin II. In contrast to B cells, T cells have proven to be difficult to maintain in culture as cell lines or clones. The use of interleukin II as a T cell growth promoter has permitted a rapidly developing technology in this area. Cultures of cloned T cells are being developed for large scale production of T cells and products for investigative use. The availability of sufficient quantities of cultured T cell clones should soon permit the isolation of sub-cellular components for biological and biochemical characterization. The elucidation of molecular mechanisms involved in recognition, restriction, and immunoregulation should be accelerated through the availability of cloned T cell lines.

The realization of the results of these fundamental studies is, for the most part, still to come. However, the few practical applications that have developed amply justify the effort and support provided to these investigations. Hybridoma technology, for example, already has supplied a variety of clinically useful reagents. The OKT series of monoclonal antibodies can effectively distinguish human peripheral blood lymphocytes on a functional or differentiated basis. Human helper and suppressor T lymphocytes can be distinguished with these antibodies and they have been employed in the clinical diagnosis of various immunologic disorders. Acute lymphoblastic leukemias have been characterized on the basis of differential expression of OKT-defined antigens and abnormal levels of T cell subpopulations have been detected in patients with multiple sclerosis and systemic lupus

erythematosus as well as in AIDS patients. Promising results with the use of monoclonal antibodies against influenza and rabies viruses have been found to detect serologic differences between isolates which were indistinguishable by previously available diagnostic antisera. Monoclonal anti-rabies antibodies have been demonstrated to be dramatically effective in protecting mice from lethal infections. Rapid, precise, and semi-automated diagnosis of human sexually transmitted diseases using monoclonal antibodies against gonococci, chlamidia, and herpes viruses will soon be readily available. It is reasonable to expect that in the near future, applications of hybridoma technology for clinical diagnosis will be extensive and will offer extremely useful approaches to therapy of a variety of immunologic, infectious, and neoplastic diseases.

Microbiology and Infectious Diseases Program

1983 Annual Report

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Microbiology and Infectious Diseases Program

Annual Report, 1982-83

Director's Report

Administrative Summary

Function and Organization: The Microbiology and Infectious Diseases Program (MIDP) supports research with the broad aim of improving health by controlling diseases caused by infectious or parasitic agents. During FY 1982-83, MIDP operated with the following organizational structure and staff.

Office of the Director (OD)

Director-William S. Jordan, Jr., M.D.

Special Assistant to the Director (International Health)-Earl S. Beck, Ph.D.

Bacteriology and Virology Branch (BVB)

Chief-Milton Puziss, Ph.D.

Virology Program Officer-William P. Allen, Ph.D.

Mycobacteriology and Mycology Program Officer-Darrel Gwinn, Ph.D.

Clinical and Epidemiological Studies Branch (CESB)

Chief-Robert Edelman, M.D.

Head, Epidemiology and Biometry Section-Richard A. Kaslow, M.D., M.P.H.

Biostatistician-William C. Blackwelder, Ph.D.

Assistant Enteric Diseases, and Clinical Trials Program Officer-

Richard E. Horton, M.D., M.P.H.

Executive Secretary, NIAID-CRS; IND Coordinator and Rapid Viral

Diagnosis Program Officer-Martha J. Mattheis, M.A.

Research Epidemiologist-Alfred J. Saah, M.D., M.P.H.

Epidemiology Associate-Anne Bailowitz, M.D., assigned for M.P.H. year
(Sept.-June) at Johns Hopkins

Epidemiologist (assigned to NIAID/AID Regional Project on Epidemiol. and
Control of Vector-Borne Diseases in the Near East)-Fred Feinsod, M.D., ScD.

Epidemiologist/Statistician (Part-time)-Patricia Koslowe, Ph.D.

Statistician-Marie Chang, B.A.

Statistical Clerk-Kathleen O'Brien, B.A.

Statistician-Marvin Godsey, Jr., B.S., (Lyme Study, N.Y.)

Medical Technologist-Barbara Goeinski, B.S., (Lyme Study, N.Y.)

Development and Applications Branch (DAB)

Chief-George J. Galasso, Ph.D.

Antiviral Substances Program Officer-Maureen Myers, Ph.D.

Bacterial Vaccines Program Officer-James C. Hill, Ph.D.

Hepatitis and Respiratory Viruses Program Officer-Franklin J. Tyeryar, Ph.D.

Influenza Program Officer-John LaMontagne, Ph.D.

Molecular Microbiology and Parasitology Branch (MMPB)

Chief-Irving Delapape, Ph.D.

Parasitology and Medical Entomology Program Officer-Harley Sheffield, Ph.D.

Special Recognition: 1) Dr. George J. Galasso received the Assistant Secretary for Health's Award for Exceptional Achievement; 2) Drs. John R. LaMontagne and

Franklin J. Tyeryar received the NIAID Director's Award in Recognition and Appreciation of Special Achievement.

Estimated Expenditures:

<u>Awards</u>	<u>Number</u>	<u>Amount</u>
Research Project Grants	922	\$ 99,089,416
Research Career Program Awards	21	816,081
Other Research Grants	15	7,338,130
Subtotal	958	107,243,627
Research Training		
Individual Awards	65	1,121,797
Institutional Awards	33	2,942,167
Subtotal	98	4,063,964
Research and Development Contracts	48	13,023,405
Interagency Agreements	3	238,122
SUBTOTAL		124,569,118
Other Objects (U.S. Japan travel, program travel, publications, workshops, etc.)		750,000
TOTAL		125,319,118

Workshops: The following workshops were organized by MIDP staff:

- January 13-----Newer Aspects in the Development of a Candidate Vaccine for Gonorrhea. Publication: Summary Report to J.I.D.
March 23-----Development of Group B Streptococcal Candidate Vaccines. Publication: Summary Report to be submitted to J.I.D.
April 5-6-----Search for Etiologic Agents in Acquired Immune Deficiency Syndrome. Jointly with IRP.
September 20-----Varicella Vaccine. Jointly with OB/NCDB and NCI.
September 28-30----Interferon (current trials; standards for production and testing). Jointly with OB/NCDB.
September 12-13----Epidemiology of AIDS. Jointly with NCI and NHBLI.
September 29-30----New and Useful Methods in Rapid Viral Diagnosis. Publication: Summary Report to JID.

Commercialization of Biomedical Research: Annual reports for the past two years made brief mention of the emerging "Academic-Industrial Complex" spawned by the application of two new technologies - recombinant DNA for genetic engineering and cell fusion for hybridoma-produced monoclonal antibodies - and its implications for faculty-university relationships. Beyond the problems posed by the recruitment of university-based scientists as corporate consultants or board members, and the engagement of faculty members in commercial ventures - problems that continue to be addressed by university presidents and corporate executives - what is happening is a dramatic example of "technology transfer." The total cost of the investment (by NIH, NSF, and others) in the basic research that makes genetic engineering possible is not known, but it was surely less than \$100 billion, the estimated commercial value of the products that industrialists hope to market. In March, 1983, it was reported that 304 firms were using recombinant DNA technology. In the past two years, certain of these corporations have promised at

least \$240 million to eight institutions through partnership arrangements variously designed to make university talent available to industry. Other universities are busy negotiating "biotechnology commercialization arrangements." To examine how universities can obtain the funding they need without jeopardizing academic freedom, more than 400 university and corporate officials organized a conference on "Partners in the Research Enterprise" in December, 1982. It had been hoped that the meeting could formulate national guidelines for university-industry interaction in developing new biomedical technology. This did not happen. Individual institutions continue to explore how best to manage the hoped-for profits from biotechnology, recognizing that there are potential conflicts of interest whenever a member of the faculty is involved with extra-university entities. NIAID-supported investigators so involved have felt it desirable to inform staff as to the nature and extent of their consultant activities. How best to protect the public interest while capitalizing on the fruits of research supported by public funds is a problem that grows ever more complicated.

Scientific Summary

The Extent of the Problem: The dramatic emergence of the Acquired Immune Deficiency Syndrome (AIDS) as a major public health problem has emphasized - with more telling effect than all past partisan protestations - the continuing importance of infectious diseases. The multitude and variety of microorganisms implicated in this syndrome, plus the presumed causative virus, are unprecedented, for never before have such defenseless human hosts existed as soil for the growth of bacteria, fungi, parasites, and viruses. Those who had considered the problem of infectious diseases solved have had to recant. Wrote Lewis Thomas (Discover/May 1983, in part).

"I used to be totally confident that the great infectious diseases of humankind were coming under control and would soon, maybe within my own lifetime, vanish as threats to human health...Just a few years ago, in an excess of hubris, I wrote an essay in which I predicted that we were nearly finished with the problems of infection, and that only a handful of still unsettled matters were left to be tidied up here and there. Moreover, I took the stand that our microbial adversaries make up a finite, short list; once done with those still at large, I saw no reason to imagine the existence of others still unknown, waiting in the wings for their turn to get at us...I take it back. We are not about to finish the job in infectious disease, nor are we likely to in the near term, maybe not in the long, long term. Legionnaire's disease and Lyme arthritis are only small hints of what might happen, unpredictably, at any time: small outbreaks of brand-new ailments turning up in small clusters of patients, carrying the threat of expanding to involve large populations... And now AIDS...There is a lot more research to be done, not just about the AIDS outbreak but about infectious disease in general. We have not yet come close to a detailed understanding of the complexities of host-parasite relationships, and we can no longer be as confident as I once was about defending ourselves against the whole world of microbes. We have not run out of adversaries, nor is it likely that we will do so for a long time to come. We need to learn a great deal more in the fields of microbiology and immunology."

NIAID/NIH and other DHHS agencies have launched a massive effort to clarify the epidemiology of AIDS, to identify the causative agent, and to develop more effective prevention and treatment. At the same time, greater attention must be paid to other infectious causes of morbidity and mortality, to wit:

- There are between 190 and 250 million acute respiratory illnesses per year in the United States, resulting in a minimum of 400 million days in bed, 125 million days lost from work and 125 million days lost from school.
- Acute gastroenteritis is the second most common illness, accounting in one survey for 9.5 % of all visits to pediatricians' offices.
- Infectious diseases result in approximately 27 million patient days of acute hospital care each year (10 % of the patient days in acute care hospitals) at an estimated direct cost of nearly \$6 billion.)
- Infectious diseases, such as tuberculosis, continue to be more prevalent in poverty areas and areas with high immigration rates. The relative risk of tuberculosis for an Indochinese refugee is 40 to 100 times greater (depending on age) than for the remainder of the U.S. public. Up to 80% of screened refugees have at least one intestinal parasite; 55%, more than one. Malaria, hepatitis and diarrheal diseases of viral, bacterial or parasitic origin, remain serious health hazards of international travel.
- In 1980 there were seven million new or recurrent episodes of genital herpes, three million chlamydial infections, three million trichomoniasis cases, two million gonorrhea cases, and 100,000 cases of syphilis. These led to one million cases of pelvic inflammatory disease, 300,000 damaged infants, 200,000 infertile women, and \$2.5 billion in direct and indirect costs.

Although the number of effective antibiotics, antivirals, and antiparasitic drugs available for the treatment of many of these infections is growing, prevention, when possible, is preferable. That is the objective of immunization.

New Vaccines The Institute's Program for the Accelerated Development of New Vaccines is well underway. Pending consideration of the independent recommendations of the Institute of Medicine which is now developing a model system for setting priorities, NIAID has targeted the following diseases for prevention by immunization with new or improved vaccines within the next 10 years:

Meningitis	Diarrhea
<u>Hemophilus influenzae</u>	Cholera
Group B Streptococci	Dysentery
	Rotaviruses
Gonorrhea	Typhoid
Bronchiolitis, croup, and	Hepatitis
pneumonia in children	Type A
Parainfluenza viruses	Type B (2nd generation)
Respiratory syncytial viruses	Chickenpox
Whooping cough	Genital Herpes
Influenza	Malaria

The status of work on these vaccines is as follows:

Hemophilus influenzae: The capsular polysaccharide of H. influenzae type b, a T-cell independent antigen, is immunogenic in children over 18-24 months of age, but does not induce protective antibodies in infants, the group at highest risk. Efforts to develop a conjugate, T-cell dependent vaccine are promising. One such vaccine, developed at the University of Rochester, consists of subunits of capsular polysaccharide chemically conjugated with either diphtheria toxoid or the

protein from a mutant designated CMR-197 (this mutant of Corynebacterium diphtheriae produces a protein which is immunologically identical to diphtheria toxin but is non-toxic). This vaccine has shown promise in clinical studies in adults and children 1-2 years of age, and testing is beginning in infants. A similar conjugate vaccine prepared by Connaught Laboratories is currently being tested in NIAID vaccine evaluation centers. A third vaccine, developed by Lederle Laboratories, uses DPT vaccine as the conjugate. A fourth, now under study by Merck, Sharp & Dohme, uses the outer membrane protein of Group C meningococci. NIAID is supporting a study at Lederle to identify the component of the pertussis cell responsible for the adjuvant effect. An efficacy trial of the most promising of these vaccines in a high risk populations has been approved.

Group B Streptococci: Two contractors, (Swenson, Temple University, and Kasper, Brigham and Women's Hospital), using somewhat different purification methods, have developed four GBS polysaccharide candidate vaccines. GBS types III (the most important), II, Ia and Ib vaccines have been prepared and the chemical and physical structures characterized. Safety tests in normal volunteers have shown that no adverse reactions occur. The vaccines elicited high titer antibodies as revealed by in vitro tests. Protection studies indicated that GBS type III serum antibody from the volunteers will protect neonatal rats against lethal challenge with type III GBS. Plans are underway for a pilot study in pregnant women to determine the antibody class elicited in the mothers and whether the level of placental antibody transferred is adequate to protect the high risk neonate.

Gonorrhea: A major objective is the development of a vaccine to prevent pelvic inflammatory disease (PID) in women infected with the gonococcus. A contractor (Buchanan, Univ. of Washington) has isolated and characterized the principal outer membrane protein (P1) of the gonococcal cell wall from strains known to cause 90-95% of PID infections in the U.S. The P1 material has been shown to be safe in human volunteers, with only minimal side reactions. The immunized volunteers produced good serum antibody responses; addition of an aluminum hydroxide gel as adjuvant further increased the antibody response. Functional assays showed the serum antibody was bactericidal. A candidate P1 gonococcal vaccine, based upon serotypes 5, 7, 8, and 9, should be effective in preventing gonococcal PID, but field trials to demonstrate efficacy will be difficult to design. Meanwhile, a pilot study is to be conducted to determine if the P1 material will elicit antibody at mucosal sites (oral, genital, and anal) and if the antibody will prevent attachment of gonococci to mucosal epithelium. A study is being planned to immunize male and female volunteers for this purpose. Only the male volunteers will be challenged with gonococci intra-urethrally to determine the protective and attachment-inhibiting activity of the anti-P1 antibody at the mucosal surface.

Parainfluenza Viruses: It had been hoped that inhibition of the fusion protein, a viral component essential to the penetration of cells by paramyxoviruses, might be a useful approach to prevention of infection by the three parainfluenza viruses. Unfortunately, methods used for other paramyxoviruses (i.e., Sendai and SV-5) have been unsuccessful with these human pathogens. Dr. Richard Compans, University of Alabama, has been attempting to isolate and purify the surface glycoproteins of parainfluenza virus type 3 for evaluation as a subunit vaccine. Efforts have been severely hampered by the inability to produce adequately purified virus preparations.

Respiratory Syncytial Virus: Much more basic information is also needed about this prevalent agent and the pathogenesis of RS disease. Efforts to develop an effective immunogen are limited to the NIAID intramural program where further

evaluation of temperature-sensitive mutants has been replaced by approaches to live vaccine construction utilizing recombinant DNA technology.

Whooping Cough: Because current whole-cell pertussis vaccine, although effective in preventing disease, is sometimes responsible for undesirable side effects, efforts are underway to develop an acellular vaccine. An acellular vaccine consisting of two major pertussis proteins has been in use in Japan for several years. NIAID has taken two approaches toward the development of a new acellular vaccine for use in the U.S.: 1) The Institute has offered the use of its vaccine evaluation units for studies of the safety and antigenicity of any candidate preparations developed by commercial manufacturers. One American manufacturer has obtained bulk vaccine from a Japanese manufacturer and plans are being made to evaluate this material. In addition, other manufacturers are working on their own acellular vaccine, and informal discussions have begun regarding future testing. 2) NIAID advertised for a contractor to develop an improved acellular vaccine; one award was made during FY 83. It is anticipated that from two to four years will be required before candidate pertussis vaccines are ready for efficacy trials. Due to high vaccine acceptance and low disease rates in this country, efficacy trials could not be done here. Informal discussions have been carried out with investigators in the United Kingdom and in Sweden about future efficacy trials in those countries.

Influenza: Clinical trials have now established the efficacy of cold-adapted (ca) vaccines during natural challenge. In three field trials, data were obtained on dose response, antigenicity, reactogenicity and genetic stability. In a comparison of the efficacy of inactivated and attenuated vaccines against experimental challenge of volunteers with a virulent, wild-type virus, the attenuated ca vaccine had a dramatic effect on the incidence of infection and illness. In contrast, while the inactivated vaccine prevented illness, it was not effective in preventing infection. Moreover, virus shedding patterns following the wild-type virus challenge were similar in the inactivated vaccine recipients to the virus shedding patterns observed in the unvaccinated, non-immune control group. Other studies conducted during the year included tests of a bivalent (H3N2+H1N1) type A vaccine in adults and children. No evidence of viral interference was detected in these studies, suggesting that the administration of bivalent and perhaps multivalent ca vaccine is possible. Further laboratory characterization of type B ca attenuated vaccines was also pursued, and two candidate master strains look promising.

A new type A master strain using an avian virus, A/Mallard/New York/6750/78 (H3N2), was developed by intramural scientists. This master strain can be used to effectively and rapidly transfer the phenotype of attenuation during reassortment with wild-type viruses. Initial clinical studies have confirmed that these vaccines are attenuated and antigenic.

Cholera: The Kaper genetically engineered strain (JBK 70) of attenuated V. cholerae (A minus, B minus) has been given to volunteers at doses of 10^6 , 10^8 and 10^{10} organisms at the Center for Vaccine Development (CVD) at the University of Maryland. Diarrhea was observed in 25% of the volunteers receiving 10^6 , 40% of those who received 10^8 , and 60% of those who received 10^{10} . Challenge studies one month later with El Tor Inaba N1 6961 demonstrated a vaccine efficacy of 90%. The development of diarrhea in volunteers inoculated with JBK 70 was unexpected since it presumably couldn't produce toxin. A new cholera toxin was found in culture supernates of this strain. Kaper may be able to delete its gene from the attenuated JBK 70 vaccine. An inactivated, oral vaccine consisting of whole vibrios and highly purified B subunits of the toxin molecule had a 64% vaccine efficacy when

vaccinees were challenged one month later with a virulent strain of V. cholerae. Challenge studies with virulent V. cholerae one month after oral immunization with yet another inactivated vaccine, procholeraagenoid (a stable high-molecular-weight aggregate of cholera toxin) combined with inactivated whole vibrios, proved this vaccine to be only 27% efficacious. Research is underway to identify other antigens of V. cholerae, such as lectins, flagellar sheath material, and outer membrane proteins, that may be important in conferring protections.

Dysentery: Formal's attenuated bivalent vaccine, a further engineered Ty2la, which affords protection against both Salmonella typhi and S. sonnei has undergone phase II protection studies at CVD. Following challenge, six of eight control subjects and three of ten vaccinees developed illnesses. An additional protection study is scheduled for November. Pending another efficacy outcome of 75%, a field study with the bivalent vaccine is anticipated by late spring in Chile and/or Israel. Formal et al have produced a genetically engineered K12 E. coli vaccine containing the plasmid from S. flexnerii type 2A that codes for controlled penetration of the intestinal mucosa, and a portion of a genome that controls production of a lipopolysaccharide that induces protective antibody. When tested in monkeys, this vaccine afforded 90% protection Phase I studies are planned in humans.

Rotavirus: The Belgium division of Smith-Kline has safety-tested the bovine rotavirus vaccine in adults and now are evaluating it in children. The results in children have not been reported yet. Wyatt et al of NIAID's LID, have several prototype vaccines under development:

- 1) New preparations of human WA rotavirus have been developed and are currently being safety-tested to be sure they are free of simian agents. Pending favorable results, a prototype vaccine will be ready for human volunteer studies this fall.
- 2) The U.K. bovine strain of rotavirus has been purified and administered to calves. This attenuated vaccine does not induce illness and has been found antigenic in calves. It has also been found safe in piglets, but does not raise antibodies. Human volunteer studies at CVD are planned by late September.
- 3) The rhesus monkey rotavirus strain isolated at the Davis Primate Center has been successfully propagated and currently is being safety-tested in monkeys to be sure it is free of any agent responsible for SAIDS.
- 4) Attenuated oral rotavirus vaccines are being developed by gene reassortment of human and animal rotavirus (bovine or simian) strains. Besides attenuation, another advantage of this technology is ease of propagation in cell culture. After thorough characterization and safety tests, at least one candidate vaccine may be ready for volunteer studies by January, 1984.

Typhoid: More experience is being gained with Ty 2la, an attenuated vaccine that has been licensed in Switzerland. Levine, Black et al have successfully immunized children in Chile. Two doses in enteric-coated capsules were used. After ten months surveillance a preliminary analysis indicates a protective efficacy of 53%. Another trial using three doses is planned.

Hepatitis A: The HM-175 strain of HAV was isolated directly by intramural scientists in primary African green monkey kidney, a cell substrate suitable for vaccine development. Tissue culture-passaged virus was fully infectious for chimpanzees but did not produce biochemical evidence of hepatitis. Similarly, HAV anti-

gen could not be detected in liver biopsies, and little or no viral antigen could be detected in acute phase stool samples. In addition, there is preliminary evidence that the tissue culture-attenuated HAV has very limited potential for horizontal transmission, at least in chimpanzees. Investigators at Merck, following up earlier studies in marmosets, have attenuated human HAV in virulence for chimpanzees by passage in FRhK6 and human diploid lung fibroblast cell cultures. A number of variants developed by passage in cell cultures showed different levels of virulence/attenuation for chimpanzees. Two chimpanzees which gave vaccine-like responses following inoculation with HAV cell culture variants were challenged with virulent HAV; both were immune to infection.

Hepatitis B: The evaluation of hepatitis B vaccine in patients of dialysis units has been completed. In this population, the vaccine was less immunogenic than in normal recipients, and efficacy was not demonstrated. Because the HBV attack rate in this trial was low, it cannot be determined that the vaccine was not efficacious, but protection against HBV events was well below 60%. The evaluation of the NIAID-developed HBV vaccine for the prevention of perinatal transmission of HBV in China is well underway. Early studies indicate that the vaccine is minimally reactogenic and reasonably immunogenic in adults and neonates.

Less expensive vaccines are being vigorously sought by both industry and NIAID. Several groups have cloned the HBV gene that codes for surface antigen in yeast as a potential source of purified antigen. Synthetic HBV polypeptides are being tested through collaborative efforts of Drs. Richard Lerner, John Gerin and Robert Purcell. Results to date have been mixed. Dr. Blaine Hollinger, Baylor College of Medicine, proposes to evaluate a promising synthetic polypeptide product in chimpanzees. A "novel" vaccine has been produced by NIAID/LBV intramural scientists Smith, Mackett, and Moss by inserting the gene that directs the production of surface antigen into vaccinia virus. The hybrid virus produced the expected local skin reaction to vaccinia virus in rabbits and stimulated antibody to HBsAg. Studies are in progress in chimpanzees. Vaccinia virus had been selected for these elegant studies because of the large size of its genome; this may be a hollow victory for the routine use of smallpox vaccine has been discontinued throughout the world.

Chickenpox: Varicella, a highly contagious viral disease of children, is not usually associated with mortality or serious sequelae. However, it is a very serious problem in immunosuppressed children such as leukemics. NIAID is currently testing an attenuated vaccine developed in Japan in this high risk population. As of June, 188 children have been vaccinated. Since a number of these vaccinees have lost antibody with time, a two-dose regimen has been adopted, the second dose being administered three months following the first. The vaccine is well tolerated, although some children develop a rash or a few vesicles after vaccination. There is some indication of a low rate of spread to susceptible siblings as evidenced by silent seroconversions. Of 17 documented household exposures in vaccinees, three developed very mild chickenpox. It appears that the vaccine will protect high risk children from serious infection and sequelae. Merck has shown a similar vaccine to be safe and immunogenic in normal children.

Genital Herpes: The complexities of the structure of herpes simplex viruses (HSV types 1 and 2) and of the latent/recurrent infections induced by them pose major challenges to vaccine development. Approaches to date include several inactivated whole virus or subunit vaccines and the engineering of a live virus vaccine. A killed whole virus vaccine produced in Europe (Lupidon) has not been subjected to controlled clinical trials; there are anecdotal reports that it has been effective

in ameliorating recurrent disease and in reducing the frequency of recurrence. Another killed virus vaccine produced in Britain has received much recent publicity, but no data are yet available on safety and efficacy in animals or on clinical efficacy. Merck (with NCI support) developed a subunit vaccine containing purified membrane glycoprotein antigens of types 1 and 2. Although ineffective in preventing recurrences of genital herpes, it is now undergoing a controlled trial among sexual partners to test its efficacy in preventing primary infection in the uninfected partner. Lederle, working with Molecular Genetics, Inc., has used recombinant DNA (rDNA) technology to produce a subunit vaccine. Watson *et al* mapped and determined the nucleotide sequence for a type 1 glycoprotein, inserted this protein-coding region into a plasmid with which they transformed *E. coli*, and obtained synthesis in the latter of an immunoreactive polypeptide related to the gD glycoprotein of the virion envelope of HSV1. Polyvalent antiserum prepared to the gD glycoprotein can recognize and neutralize infectivity of both HSV 1 and HSV 2.

A live virus vaccine is the goal of Dr. Bernard Roizman (University of Chicago) who has selectively created live mutants of HSV by rDNA technology seeking to delete genes contributing to neurovirulence and reactivation from latency. Some of these mutants are being tested in mice and monkeys for efficacy in protection against primary and recurrent disease (but not necessarily against infection).

Malaria: The most promising, and the most advanced, approach to a malaria vaccine is the development of an antisporozoite vaccine. Antigen having a molecular weight of 44 kilodaltons isolated from the surface of sporozoites of Plasmodium berghei stimulates production of an antibody which abolishes infectivity when it is incubated with sporozoites. This antibody also blocks entry of sporozoites into cultured cells and confers passive protection on recipient mice. Similar results have been obtained with P. knowlesi and P. cynomolgi in primates. Recently, in studies with P. falciparum, a human malarial species, Dr. Ruth Nussenzweig and colleagues (New York University) have identified a protective surface antigen. Monoclonal antibodies raised against P. falciparum sporozoites reacted with circumsporozoite proteins uniformly distributed over the sporozoite surface. Epitopes recognized by the monoclonal antibodies were expressed on sporozoites from different geographical isolates of homologous species but were not detected on sporozoites of heterologous species nor on blood forms of the parasite. Monoclonal antibody specifically immuno-precipitated two polypeptides from extracts of P. falciparum sporozoites. Extracts were also immunoprecipitated with the serum of a human volunteer successfully vaccinated with sporozoites of P. falciparum. Incubation of the appropriate monoclonal antibody with viable sporozoites of the homologous species significantly reduces their infectivity as shown by sporozoite neutralization assays carried out in splenectomized chimpanzees.

In addition, the group at NYU, working in collaboration with the NIAID Laboratory of Parasitic Diseases, has prepared mRNA from P. knowlesi and constructed a cDNA library. A clone that expresses sporozoite surface antigen has been isolated from this library. This is the first potentially protective malarial antigen to be cloned by recombinant DNA technology. Although much progress has been made, an effective vaccine will not be available in the near future. Since one sporozoite can initiate an infection, the vaccine must be 100% effective. At the present time it is not known whether antigens produced by recombinant DNA techniques will be highly immunogenic. It is probable that the ideal vaccine will be one which combines an antisporozoite antigen with one which induces antibody to merozoites. The major drawback with such antigens is that they must be administered with an adjuvant and, as yet, there is no adjuvant suitable for human use.

Research Capsules

Biotechnology: Monoclonal antibodies have been successfully used for the identification of gonococcal, chlamydial and herpesvirus infections in direct diagnostic tests requiring only 15 to 20 minutes.

Elegant cytohybridization techniques now make possible the detection of virus genes and their products in human tissues (e.g., ganglia), facilitating the study of latent/persistent infections.

The automated microsequencing of proteins can now be carried out at the 5-to 10-picomoles level on polypeptides obtained from one- and two-dimensional gel electrophoresis.

Relatively short synthetic peptides that mimic part of a protein sequence elicit an antiserum that reacts with the partially mimicked microbial protein. Such peptides may serve as the basis for synthetic vaccines.

Plasmids have been constructed to direct the synthesis of three human interferons in yeast.

Cryptosporidiosis: Cryptosporidium, primarily a coccidian parasite of calves and other animals, has joined another parasitic protozoan, Pneumocystis carinii, as a major cause of opportunistic infections in patients with AIDS. Infection of the intestinal tract is manifest by abdominal cramps and diarrhea. No specific drug treatment is available. Recently, self-limited illnesses have been observed in immunocompetent persons who had direct contact with the feces of infected calves.

Papilloma viruses: Information is rapidly accumulating about the 18 types of human papilloma virus (HPV) identified to date. Types 1 and 6 have been related to plantar warts, types 3, 5, 8, 9, 12, 14, and 15 to epidermodysplasia verruciformis, and types 6 and 11 to genital and laryngeal warts. Gene sequences homologous to types 11 and 16 have been detected in women with cervical cancer, with sequences for type 16 being most prevalent. NIAID has initiated controlled clinical trials of interferon in children with laryngeal papilloma and in adults with condyloma acuminatum.

Toxic Shock Syndrome: Lysogeny (integration of bacteriophage DNA into the bacterial genome) has been demonstrated in 11 of 12 strains of Staphylococcus aureus isolated from patients with TSS. Only one of 18 strains not associated with TSS showed the presence of bacteriophage. The investigators suggests that TSS toxin production is phage-dependent. Other investigators were unable to produce TSS in menstruating rhesus monkeys by direct vaginal inoculation of toxin-producing strains recovered from the vaginas of women with TSS.

Syphilis: Two achievements will facilitate studies of immunity to syphilis: successful cultivation of Treponema pallidum has been confirmed, and subculturing appears promising; a clone bank representing the entire genome of T. pallidum has been constructed.

International Health

International health research programs are administered by Dr. Earl Beck, Special Assistant to the Director, MIDP, in conjunction with individual program officers.

International Collaboration in Infectious Diseases Research (ICIDR) is divided into Part A, International Program Project Grants (POIs), and Part B, International

Exploratory/Developmental Research Grants (R21s). The research emphasis of the ICIDR program is infectious diseases and the immunology of these diseases, and includes, filariasis, leishmaniasis, leprosy, malaria, schistosomiasis and trypanosomiasis. The P01s of Part A are broadly based multi-disciplinary research programs with a well-defined central focus or objective, and with the major portion of the research (80%) being conducted overseas with a foreign collaborator in a developing country.

The R21s of Part B are small grants designed to encourage an individual U.S. investigator to develop a biomedical research program with an overseas collaborator, with the major portion of the work performed in a developing country. Essentially, these are planning types of grants with the opportunity of developing into a P01, an R01 or a foreign research grant.

The ICIDR Program was initiated in September 1979 with the awarding of P01s to Harvard University (AI 16305) with collaborators in Brazil; to Michigan State University (AI 16312) with affiliates in Sudan, to Cornell University (AI 16282) with research associates in Brazil; to the University of Illinois (AI 16308) with collaborators in Thailand; and to Tulane University (AI 16315) with overseas counterparts in Colombia. These five program project grants will start their fifth and last year in 1983 under the initial award made in September 1979.

There were five R21 grants made to the following: Columbia University (R21 AI 16314) with three years of support to work with Brazilian researchers; Ohio State University (R21 AI 16285) with three years of funding to collaborate with Nigerian associates; University of South Florida (R21 AI 16287) with three years of support to work with Brazilian investigators; University of Hawaii (R21 AI 16273) with four years of funding to collaborate with Indian researchers; and the University of Washington (R21 AI 16290) with five years of support to develop a collaborative program in Mexico. The latter is about to start the last year of research under the initial 1979 award; all of the other grants have terminated according to the stated years of support.

When plans were made to readvertize the RFA for the ICIDR program, it was decided that the applicants needed at least a year to establish some rapport with their foreign colleagues, to prepare cooperatively an acceptable grant proposal, and to obtain the required approvals from the host country. In the first round, approximately eight months elapsed between publication of the RFA and receipt of applications, and this did not appear to give the applicants sufficient time to prepare an application and obtain the required approvals. In addition, program staff felt that for the competing P01 grantees, a time frame should be established so that anyone who might not compete successfully would have the fifth and final year in which to phase out the program or obtain alternate funding. The RFA was revised to exclude the requirements that the research be done in a "developing" country. Instead, the research was to be performed in any country that had a tropical disease problem(s) of significant health importance. Another modification placed a dollar limit of \$350,000 total cost on a P01 and \$50-60,000 tagged to an R21. Unfortunately, the latter limit was too low when compared with the average cost of an R01. The cost of these grants was specified to stretch a limited budget to fund more grants and involve a larger number of U.S. and foreign investigators in international health research. The RFA was published in November 1981, with a receipt date for proposals on October 15, 1982. Twenty program project proposals were received from U.S. investigators with overseas collaborators in 15 different countries. There were some country duplications, with Brazil being the most popular; four investigators had chosen collaborators in this country. All of the

five current POls competed; only one received a priority score which fell beyond the payable range. Of the 20 grants reviewed, 13 were approved and seven were disapproved. Staff has requested support for three more POls which will make a total of seven. There were only two more Exploratory/Developmental Grants (R2ls) received than POls. This was unexpected based on the experience of the first advertisement when the ratio was nearly three to one in favor of the smaller grant. Of the 22 R2ls received and reviewed, 19 were approved while three were disapproved. Fourteen different countries were named in the grant proposals, with Brazil and Venezuela being mentioned three and four times, respectively. For administrative purposes, the R2ls were reclassified as R0ls; to date, three have been funded, and it is anticipated that another four will receive support in FY84. The reviews of the two types of grants were held separately to lighten the workload for the reviewers, and this arrangement appeared to be a very demanding and arduous assignment. It is also interesting to compare the number of approvals to disapprovals for the first (1979) and second (1983) reviews. In the first, five of 14 POls and 23 of 39 R2ls were disapproved. In the second review, only seven of 20 POls and three of 22 R2ls were disapproved. Although there could be any number of factors that might have influenced the outcome, one factor that could have increased the quality of the grant proposal was the increased time for preparing the application, 12 months instead of 8 months. The lead-time might be increased further to provide greater opportunity for interaction between U.S. scientists and their foreign collaborators. A number of applicants indicated that the preparation time could have been longer.

In general, the POls are performing well as indicated by their publication record and by personal contacts at meetings and through telephone conversations. The program officer did not make any site visits this year.

Only one of five of the initial R2ls applied for a POl. Although this was somewhat disappointing, it appears from reports of U.S. investigators and their overseas collaborators that the scientific interactions were productive. An evaluation of this program on such a small sample size would appear to be premature. Perhaps, after a second round of grants, an evaluative trend will be more evident.

The U.S.-Japan Cooperative Medical Science Program: This program has provided the basis for collaborative research between scientists of the two countries since its inception in 1965. Research is focused on diseases of health importance to people of Asia, although some of the diseases occur in populations throughout the world. The following research areas are included in the program: cholera, environmental mutagenesis and carcinogenesis, hepatitis, leprosy, malnutrition, parasitic diseases, tuberculosis, viral diseases, and immunology, the latter being discipline rather than disease-oriented.

A symposium entitled, "The Current Status of New and Unresolved Problems in Parasitic Diseases," was held at the National Institutes of Health in Bethesda, Maryland, on July 18 and 19, 1983. This symposium was initiated by the Joint Committee to review the problems and research opportunities in the parasitic diseases field prior to updating or expanding the current guidelines of the Parasitic Diseases Panels. A very successful two-day program included presentations on schistosomiasis, filariasis, onchocerciasis, amebiasis, giardiasis, pneumocystis pneumonia, cryptosporidiosis, strongloidiasis, malaria, trypanosomiasis, and leishmaniasis. The Committee listened to recommendations made by the Joint Parasitic Diseases Panels and referred the matter for discussion at the next meeting of the Subcommittee on Program Review and Planning to be held in February 1984. The recommendations of the Subcommittee will be considered at the next Joint Committee meeting in July 1984.

BACTERIOLOGY AND VIROLOGY BRANCH

The mission of the Bacteriology and Virology Branch is development and support of a wide array of programs in biomedical research; these include program project grants, individual research grants, career development awards, training programs of both institutional and individual postdoctoral fellowships, and contracts for targeted research in the fields of bacteriology, virology, and mycology. The Branch is headed by Dr. Milton Puziss, who also administers the Bacteriology Program. The Virology Program of the Branch is headed by Dr. William P. Allen, who also serves as Executive Secretary of the Panel on Viral Diseases of the U.S.-Japan Cooperative Medical Science Program. The Mycology and Mycobacteriology Programs are administered by Dr. Darrel D. Gwinn, who also serves as Executive Secretary of both the Panel on Leprosy and on Tuberculosis of the U.S.-Japan Cooperative Medical Science Program.

Approximate Level of Support

Bacteriology and Mycology

<u>Activity</u>	<u>Number</u>	<u>Amount</u>
Research Grants	167	\$15,768,626
Research Program Projects	6	2,890,733
Research Contracts	7	784,010
Career Awards	6	222,311
Training Grants	12	1,104,754
Fellowships	7	128,463
 Total	205	\$20,898,897

Virology

Research Grants	170	\$20,071,265
Research Program Projects	2	832,127
Research Contracts	-	-
Career Awards	11	408,854
Training Grants	9	1,038,030
Fellowships	16	304,526
 Total	208	\$22,654,802

Branch

Total	<u>413</u>	<u>\$43,553,699</u>
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Program Summary

The programs of this Branch focus on projects of both fundamental and practical biomedical research; the ultimate objectives are translation of new knowledge into better methods for the diagnosis, prevention, and therapy of bacterial, viral, and fungal diseases. Certain programs, as those on sexually transmitted diseases, genital herpes, hospital associated infections, and most recently, acquired immune deficiency syndrome, are also of significant interest to the Congress. Research programs on the viral diseases of rabies, dengue, arthropod

transmitted viruses, leprosy, and tuberculosis, are major projects of interest to the U.S.-Japan Cooperative Medical Science Program.

Bacteriology

Research Highlights

Sexually Transmitted Diseases

2 P01 AI 12192-08 K. K. Holmes (University of Washington): This complex project involves study of a variety of sexually transmitted disease problems. In one subproject, monoclonal antibodies were developed for serogrouping of gonococcal strains. Using these antibodies, it was found that gonococcal strains isolated from homosexual males had the mtr gene with greater frequency than did strains from heterosexual patients. The mtr gene confers upon the gonococcal strain resistance to hydrophobic molecules, including fecal lipids; this would provide the strain with a protective mechanism to resist toxicity of these agents and the ability to colonize the rectum. In another study, characterization of a cryptic plasmid found in Chlamydia trachomatis is underway; a detailed genetic map of one plasmid found in E. coli has been constructed. A variety of clinical isolates of C. trachomatis examined thus far possess this plasmid. In other studies, a new method of directly detecting chlamydia infected cells was developed, based on a monoclonal antibody highly specific for the chlamydial antigen, coupled to a fluorescein dye for direct immunofluorescent microscopic detection. This method now makes possible a rapid, specific, and relatively inexpensive visual test for the presence of chlamydia in cervical and urethral secretions that have been stained for immunofluorescence. The risk of chlamydial infections in males and females was much higher in younger age groups. In women, an estimated 50 percent of chlamydial infections were asymptomatic and only detected by screening. A cost-effectiveness study for such screening is needed. Other data indicate that prostatic inflammation or infection may be an important cause of persistent nongonococcal urethritis in patients not responding to drug therapy.

5 P01 16959-03 J. C. Hume (Johns Hopkins University): In this STD research unit, data from a subproject on human genital papilloma virus (HPV) have shown possibilities for diagnosis of infection by examination of Pap smears for viral antigen by an immunoperoxidase staining technique. Correlation between staining of Pap smears and direct examination of cervical tissues was about 80 percent. In studies on tissues from human laryngeal papillomas, HPV type 6 was identified. A number of HPV subtypes are recognized; subtype C is the most prominent in extension of this disease to extra-laryngeal sites. Infection with HPV6 type C is possibly indicative of a poorer prognosis. As the virus of juvenile laryngeal papilloma is transmitted to the fetal larynx during passage through an infected birth canal, difficult labor and other factors that increase the contact period would tend to increase the risk of occurrence.

2 R01 AI 16692-04 M. V. Norgard (University of Texas, Dallas): Dr. Norgard has constructed a clone bank representing the entire Treponema pallidum (Nichols) genome in E. coli RR1. Results using recombinant DNA technology suggest that E. coli may be capable of expressing virtually any important T. pallidum antigen (immunogen) gene, thus providing an advance in our ability to characterize and isolate the immunodominant antigens of this spirochete involved in protective immunity in the rabbit model of syphilis. Other data showed that T. pallidum antigens synthesized by the E. coli clones allowed for the partial purification

and initial characterization of these treponemal antigens. A large array of anti-*T. pallidum* hybridomas producing monoclonal antibodies against the organism was constructed. These monoclonal antibodies have now become an important analytical tool of great potential in the study of syphilis infections.

7 R01 AI 20006-01 S. J. Norris (University of Texas, Houston): By building on the late Dr. Howard Fieldsteel's studies on attempts to culture *T. pallidum* in vitro, Dr. Norris has achieved considerable success towards this long sought goal. He reports that Fieldsteel's tissue culture cell line (cottontail rabbit epithelium - Sf1Ep) is most useful because it is a slow growing cell. Mean maximum yield was 81×10^6 treponemes per culture, an average of 16 fold increase. Fetal bovine serum fraction was necessary, together with a specific nutrient medium that reflected a requirement for fatty acids. Irradiated Sf1Ep cells also were capable of supporting treponemal growth, indicating that tissue culture cell multiplication was not an absolute requirement; rapidly growing cells were poor growth supporters because of the possible creation of toxic conditions. Initial attempts at subculturing the treponemes from primary cultures also appear promising. If successful, this study will have great potential for producing *T. pallidum* in quantity for development of a candidate syphilis vaccine, as well as for expanding our understanding of the major antigens involved in the host immune response to syphilis.

Streptococcal Infections and Sequelae

5 K04 AI 00323-04 C. J. Baker (Baylor College of Medicine): This investigator has been studying the immune responses to neonatal Group B streptococcal (GBS) infections. The adherence of GBS cells to human epithelial (buccal) cell surfaces in infants infected with GBS was considerably greater than in healthy matched controls. The data further suggest that infants at risk for invasive GBS infection may have increased receptor sites for certain GBS strains. In other studies, the bactericidal activity of adult and neonatal sera was antibody independent and may proceed by either the classical or alternative complement pathways. Data from a long-term follow up study of neurologic sequelae in infants who had GBS meningitis are being evaluated in children who are now three to nine years post-GBS meningitis; 64 percent are normal, 14 percent have global mental retardation and cortical blindness associated with a developmental level of about 10 months, and the remainder have mild abnormalities such as spastic monoparesis or school problems.

5 R01 AI 10085-19 E. H. Beachey (University of Tennessee): Dr. Beachey and co-workers are investigating the protective immunity evoked by a chemically synthesized peptide fragment of Group A streptococcal (GRAS) M protein. The synthetic peptide (S-CB7), of 35 amino acids, was derived from cyanogen bromide cleavage of streptococcal M protein. The S-CB7 was highly immunogenic in eliciting protective antibody against the type 24 GRA streptococcus. Lymphocytes from the immunized rabbits responded to heterologous types 5 and 6 streptococcal M proteins as well as the homologous type 24, suggesting that cellular immunity is directed against a common M protein structural determinant. Indications are that only small segments of the M protein are needed to evoke protective immunity. Other data suggested that the available monoclonal antibodies against M proteins may be used to map the protective, and perhaps the tissue cross-reactive determinants of streptococcal M proteins.

5 R01 AI 16624-04 J. K. Spitznagel (Emory University): Dr. Spitznagel and co-workers have completed a study of the clinical aspects of arthritis due to GBS in a rat model. Pathology studies show that streptococcal antigen in rat joints tends to be associated with granulocytic infiltration in acutely inflamed soft tissue. Arthropathogenicity of whole GBS cells is essentially intermediate between that of Group A streptococci, which fail to produce disease, and Group D streptococci, which produce disease more quickly and of much shorter duration than disease due to Group B streptococci. Group A streptococci are very resistant to lysozyme, Group D are very sensitive, and GBS are intermediate. Heat killed whole GBS type III cells induced rat arthritis with a lag period of 7 days, whereas sonicated bacteria induced similar effects in 24 hours. Evidence suggests that the alternate complement pathway may be involved in the inflammatory response to bacterial cells; host lysozyme was also shown to process the streptococcal cells in vitro to a potentially more toxic form. These studies have contributed further to an understanding of experimental inflammatory responses to GBS.

Hospital Associated Infections

2 R01 AI 19046-02 R. A. Garibaldi (University of Connecticut Health Center): This project focuses on the need for a better understanding of the pathogenesis of postoperative infections. A striking association was shown between infectious complications and age, surgery duration, and severity of underlying disease. The class of surgical wound correlated with the acquisition of postoperative wound infection: e.g., male, preoperative stay and operative site correlated with postoperative respiratory infections, and female, surgical site correlated with post operative urinary tract infection. Accepted guidelines have been widely published for parenteral antibiotic use as prophylaxis against postoperative surgical infections. Despite these guidelines, many surgeons do not adhere to the recommendations. Unnecessary, prolonged or inappropriate antibiotic usage, according to the study data, has tremendous economic impact on excessive hospitalization costs. More importantly, patients who received inappropriate prophylaxis were more likely to acquire infection with antibiotic resistant bacteria. Microorganisms such as S. aureus, enterococci and enterobacter species were the predominant offenders. Study of intraoperative wound cultures, it is believed, is an excellent method to identify high-risk patients.

5 R01 AI 10108-13 A. I. Braude (University of California, San Diego): This investigator and co-workers have been studying the effect of E. coli J5 antiserum for therapy and prophylaxis of gram-negative bacteremia in immunocompromised patients. Studies are now underway in patients with bone marrow transplants and in leukemia patients to determine the prophylactic effectiveness of the J5 antiserum. IgM is the protective immunoglobulin against bacteremia, but it was found to have a very short half-life. Monoclonal antibodies to E. coli -0111 LPS and to the J5 LPS were generated. The anti-0111 and the anti-J5 monoclonal antibodies protected in vivo by preventing death in mice from 0111 LPS and the dermal Shwartzman reaction in rabbits, and in vitro by decreasing the B cell proliferation response to 0111 and J5 LPS. These studies with monoclonal antibodies to several LPS determinants offer a useful tool for unraveling the mechanism of protection by core LPS antiserum in man.

Cystic Fibrosis

5 R01 AI 15835-04A1 G. B. Pier (Brigham and Women's Hospital): A vaccine to prevent the severe effects of chronic lung infection in cystic fibrosis patients has long been needed. Dr. Pier has investigated the human immune response in volunteers to vaccination with a high M.W. polysaccharide from a Pseudomonas aeruginosa immunotype (IT-1). Levels of specific immunoglobulin to IT-1 in the volunteers remained significantly elevated 21 months post-immunization. Several of the individuals responded to the IT-2 and IT-5 serotypes, but none responded to serotypes IT-3, 4, 6, or 7. Immune serum IT-1 specific antibody was of the IgG, IgA, and IgM classes. Purified serum IgG and IgA mediated high levels of killing of P. aeruginosa by peripheral blood leukocytes; complement potentiated the IgG-mediated bactericidal effect but had no effect on IgA mediated bacterial killing. The data suggest that the P. aeruginosa polysaccharide vaccine induces a long lived, serotype specific immune response. Opsonophagocytosis of the pseudomonas by serum IgA and mononuclear cells suggests that immune IgA and monocytes may be an important protective mechanism for the immunocompromised host.

5 R23 AI 18215-02 K. B. Adler (University of Vermont): This young investigator has established that P. aeruginosa elaborates a product that stimulates secretion of mucin by explants of both human and rodent trachea in organ cultures. The heat-stable stimulatory effect is not related to the mucoid character of the bacterial isolates nor their serotype. Current data show that stimulation of mucin secretion by the bacterial filtrates may involve binding to ganglioside receptors on the cell surface, prior to stimulation of secretion. Calcium ion influx also appears to be a component of the stimulatory effect; the stimulatory component is not a pseudomonas elastase. It is also possible that prostaglandin E could be acting as an inhibitor of mucin secretion stimulation.

Legionnaires Disease

AI 17815-03 B. H. Iglewski (Oregon Health Sciences University): This investigator earlier found that Legionella pneumophila (LP) produced an exotoxin of unknown properties. She now reports that the colorless cytotoxin is produced in good yield in a chemically defined medium; has a M.W. of 800, is heat stable and resistant to acid, alkali and enzymatic action. One microgram is toxic for Chinese hamster ovary cells in culture. Other data show that the toxin adversely affects the ability of PMNs and human monocytes to activate their oxygen-dependent antimicrobial systems. The ability of LP to withstand the phagocytic-antimicrobial action of these antimicrobial systems and survive intracellularly most likely is related to the depression of these systems by the Legionella toxin. The toxin may therefore play a significant role in the pathogenesis of L. pneumophila infections.

AI 17254-03 M. A. Horwitz (Rockefeller University): Earlier reports from several investigators showed that patients with Legionnaires' disease (LD) develop cell-mediated immunity to Legionella pneumophila (LP). Recent data imply a limited role for humoral immunity and a major role in host defenses for cell-mediated immunity. Electron microscope (EM) study by Dr. Horwitz showed that the LP inhibits phagosome-lysosome fusion, and that erythromycin at concentrations completely inhibiting intracellular LP multiplication had no effect on this fusion process. Other EM studies revealed that the organism is phagocytized by a

highly unusual process in which a monocyte pseudopod is coiled around the bacterium as it becomes internalized. Human monocytes, alveolar macrophages, and PMNs all phagocytize the LP organism by this unusual process. The live LP resides within the vacuoles containing other bacteria. Activation of the monocytes does not influence the pH of the LP vacuole; this may help to understand the mechanism by which LP survives and multiplies in host cells.

Staphylococcal Infections

RO1 AI 14998-04 M. E. Melish (Kapiolani-Children's Medical Center): Dr. Melish has been studying staphylococcal epidermolytic toxin (ET) because of its similarity to toxic shock syndrome and the scalded skin syndrome of infants. Her recent data show that specific binding of ET occurs on membrane fractions of human and murine skin; this binding is reversible and has a requirement for Cu and Mg ions. The heat stable and heat labile forms of ET are chemically and antigenically distinct. Receptor activity on membranes from the dermis as well as the epidermis demonstrate that receptors are not limited to all of the upper epidermis, the site of action of the toxin. ET from streptococci also has been isolated and purified; it has a M.W. of 30,000 daltons, differs chemically and physically from all previously described streptococcal toxins, and is produced by a majority of Group A beta-hemolytic streptococci. It is also found free in blister fluid from patients with streptococcal cellulitis and in blister fluid from a mouse model. The mechanism of action and location of binding sites is not yet clear.

AI 17242-03 B. A. Sanford (University of Texas, San Antonio): This project is concerned with identifying the macromolecules responsible for the interaction of bacterial respiratory pathogens with virus-infected mammalian cells (bacterial superinfection). The experimental model involves canine kidney cell cultures infected with various strains of influenza A virus that are then exposed to radio-labeled bacterial pathogens - Staphylococcus aureus or Group A or B streptococci. In summary, Dr. Sanford's data show that two distinct mechanisms may be involved in which influenza A virus-infected cells can promote the adherence of bacterial respiratory pathogens in vitro. First, virus infection induces the appearance of cell membrane receptors that interact with bacterial adhesins. Second, virus infection induces the appearance of membrane receptors that interact with fibrinogen that can, in turn, cross-link certain bacteria to the cell surface. These mechanisms, if found to occur in vivo, could explain the observed association between influenza A virus infection and the subsequent bacterial superinfection with S. aureus and Group A streptococcus.

Basic Research

5 RO1 AI 15244-05 W. E. C. Moore (Virginia Polytechnic Institute): Dr. Moore is developing a method for rapid identification of clinical bacterial isolates based on analysis of electrophoretic protein patterns of bacterial cultures. Current data show that these patterns from a very broad range of bacteria provide invaluable confirmation of identification by extensive classical biochemical tests. In some instances, the patterns distinguished genetically unrelated species that cannot be differentiated by conventional biochemical tests. Computer programs to accept laser scans of the electrophoresis gels are now operative in the investigators laboratory; initial computer programs have already been written for comparisons of "unknown" strains with reference strains.

Development for wide clinical application will depend on computer pattern recognition. Although incomplete, the data are encouraging for increasing evidence and documentation that a reliable, rapid and inexpensive diagnostic procedure can be developed.

Leprosy

N01 AI 22682 P. J. Brennan (Colorado State University, Fort Collins): Dr. Brennan has continued to characterize the species-specific glycolipid antigens of Mycobacterium leprae. Upon "dissecting" the glycolipid-I molecule, he found that the lipid core was devoid of serological activity and the trisaccharide entity was the serological determinant. This trisaccharide has been chemically synthesized in the laboratory and coupled to bovine serum albumin. The synthetic antigen is highly reactive (ELISA test) with leprosy patients' sera, but shows no activity with normal human sera, or that from tuberculosis patients or from patients infected with atypical mycobacteria. This antigen is of value in the serological diagnosis of leprosy; if the antigen is protective, it also may be useful in the development of an effective leprosy vaccine.

R22 AI 19302-01 B. J. Gormus (Tulane University, Louisiana): Dr. Gormus found that adult sooty mangabey monkeys inoculated with M. leprae (human origin) developed skin lesions and other symptoms identical to human leprosy; they also required drug therapy to control the disease. This should be an extremely useful primate model to study human leprosy. Additionally, Dr. Gormus has determined that both rhesus and African green monkeys are also susceptible to infection with M. leprae. These species should also be valuable models for understanding human leprosy, since rhesus and African green monkeys are more readily available than mangabeys. Other laboratories worldwide now may be able to use these models in the study of leprosy in nonhuman primates.

Tuberculosis

R22 AI 18357-03 P. J. Brennan (Colorado State University, Fort Collins): Dr. Brennan has discovered a new and unique group of mycobacterial glycolipid antigens on the surfaces of M. kansasii, M. fortuitum, M. flavescens, M. xenopi, and M. terrae. They are all highly antigenic and species specific (ELISA test) and hence are ideally suited for the serodiagnosis of human mycobacterial infections. Dr. Brennan believes that these substances represent a new concept in mycobacterial surface chemistry and antigenicity, whereby some "rough" species contain merely the trehalose-containing "core" while in other highly immunogenic "smooth" species, the core is garnished by a plethora of species specific sugars. The key questions now are whether or not M. tuberculosis and M. leprae are part of this pattern, and can their specific trehalose-containing lipooligosaccharides be used for the serodiagnosis of tuberculosis and leprosy.

R22 AI 17812-02 Q. N. Myrvik (Wake Forest University, Winston-Salem, North Carolina): Dr. Myrvik has shown that live M. bovis (BCG) and live M. tuberculosis (H37Rv) caused disruption of the phagosomal membranes of normal rabbit alveolar macrophages (AM) within 24 hours following phagocytosis *in vitro*. At least 25 percent of the intracellular BCG and 50 percent of the intracellular H37Rv were found free in the cytoplasm at the 24 hour interval. In contrast, M. tuberculosis (H37Ra), M. phlei, and M. smegmatis could not alter the phagosomal membrane in normal AM, since they were consistently seen within intact phagosomes

and exhibited various degrees of morphologic disintegration. This evidence indicates that the pathogenic capacity of mycobacteria is somehow linked to this membrane-disrupting virulence mechanism.

Mycology

P01 AI 16252-05 (Project VII) L. S. Young (University of California at Los Angeles): Dr. Young's studies have been directed toward the identification, isolation and characterization of certain protein antigens of Candida albicans that circulate during invasive disease. He has determined, using an isoelectric focusing and a blot technique with various sera, that essentially all the proteins within a cytoplasmic protein extract of C. albicans are immunologically recognized by the serum IgG of 30 different patients with documented invasive disease. Also, there is no immune recognition by pooled normal serum samples. He has isolated three selected proteins by preparative isoelectric focusing and has recently produced mouse monoclonal IgG to one of the antigens. He intends to use the monoclonal antibody both as a reagent in a standardized enzyme immunoassay and as a tool in affinity chromatography to prepare highly purified protein antigen.

R01 AI 14387-05 J. M. Becker (University of Tennessee): This investigator is developing drugs to use in the treatment of Candida albicans infections. Even though the literature contains numerous references that the polyoxins are not highly active *in vivo* against C. albicans, Dr. Becker has demonstrated that polyoxin D at millimolar concentrations caused marked morphological alterations of both the yeast and hyphal forms of C. albicans. To pursue this lead further, he synthesized eight analogs of polyoxin L from uridine. All of these analogs inhibited chitin synthetase from C. albicans and caused severe morphological distortions of this yeast in culture; a number of the analogs killed C. albicans at millimolar concentrations. These results suggest that chitin synthetase inhibitors may have a potential as anticandidal drugs.

Virology

Genital Herpes: The Institute funded a new program project research grant on the epidemiology and natural history of genital herpes, awarded to Emory University under the direction of Dr. Andre Nahmias. Another grant project, to Dr. Lawrence Corey (University of Washington) on the natural history of genital herpes, was expanded to include studies on factors contributing to the transmission of the virus from patients to their sex partners.

R01 AI 14341-04 L. Aurelian (Johns Hopkins University): Recurrent episodes of herpes are associated with certain changes in cell mediated immune (CMI) responses of the patients. Dr. Aurelian has been defining and characterizing regulatory events of CMI as they correlate with the development of clinically symptomatic recrudescent lesions caused by herpes simplex virus (HSV). Recently completed studies have shown that peripheral blood leucocytes (PBL) from patients with a history of recurrent genital herpes disease display normal lymphoproliferative (LF) response but impaired lymphocyte inhibitory factor (LIF) production at prodrome and recrudescence. The impaired LIF responses correlate with the presence in PBL of a significantly increased proportion of suppressor T cells. Cell depletion studies using monoclonal antibodies and complement indicated that when PBL of recrudescent patients were depleted of either suppressor T cells,

macrophages or immune adherent cells, they regained their ability to enhance natural killer cell activity and LIF activity. The findings suggest that natural killer enhancing activity and the LIF are actively suppressed during recrudescence. Suppression requires the presence of T8 suppressor cells and macrophages. This suppression of immune cells may help to explain why some individuals get recurrent herpes infections while others do not.

R01 AI 19257-01 L. P. Pereira (State of California Department of Health Services): Cytomegalovirus (CMV) is a herpesvirus that is a frequent cause of interstitial pneumonia in immunosuppressed hosts. Rapid determination of infection is critical to the management of these patients. Current tests for CMV were unreliable. Dr. Pereira greatly improved the reliability by employing highly specific monoclonal antibodies for detection of CMV antigens in biopsied lung tissue. In a small series of specimens tested, the sensitivity and specificity of the test with monoclonal antibodies for the rapid diagnosis of CMV infection was 100 percent. A mixture of nine monoclonal antibodies was superior to the use of any one monoclonal antibody.

R22 AI 18085-01 N. Nathanson (University of Pennsylvania, Philadelphia): The molecular mechanisms of viral pathogenesis are being investigated by Dr. Nathanson, with emphasis on viral virulence using monoclonal antibodies and variants of human viruses that differ in their ability to cause disease in mice. Two viruses, La Crosse (LAC) and Tahyna (TAH) differ markedly in their ability to kill suckling mice when inoculated below the skin. The difference has been found to lie in the ability of the more virulent virus, LAC, to replicate in striated and cardiac muscles whereas the avirulent TAH does not. The gene(s) that control this virulence are being identified and characterized. The middle-sized of three RNA segments that make up the virus genetic material encodes for this virulence. When this mid RNA segment is exchanged for a similar one from an avirulent strain of virus, the ability to kill suckling mice is lost. Also, in order for the virus to infect susceptible cells it has been shown that the viral lipid envelope must fuse with the cell membrane. This fusion occurs only at a low pH and appears to be controlled by the molecular composition of the virus envelope. Knowledge of these virulence mechanisms may lead to a better understanding of viral pathogenesis and control of disease.

R22 AI 17995-02 L. Rosen (University of Hawaii): The four dengue viruses as a group are considered by far the most important viruses transmitted to man by mosquitoes, whether measured in terms of the number of human infections or the number of human deaths. Present knowledge is insufficient to explain the persistence of these viruses before humans lived in large groups in close proximity to one another. Dr. Rosen's studies of mechanisms of viral persistence have revealed the existence of a species of mosquitoes, Aedes (Gymnotopha) mediovittatus, that is a better candidate for perpetuating the virus than the notorious vector Aedes aegypti. Ae. mediovittatus is found throughout the Caribbean area of the Western Hemisphere wherever dengue is endemic. The dengue viruses have been shown to be passed from one generation of mosquitoes to the next by a process called transovarial transmission. In Ae. mediovittatus the efficiency of transmission from female mosquito to offspring was shown to approach 9 percent, greatly exceeding that of Ae. Aegypti. Thus, Dr. Rosen has demonstrated that dengue endemicity can be explained by perpetuation of the virus in mosquitoes from generation to generation and that the transmission rate in Ae. mediovittatus is the most efficient of any flavivirus observed thus far.

R22 AI 09706-12 H. Koprowski (Wistar Institute, Philadelphia): Human diploid rabies vaccine, developed by the Wistar Institute, and produced by Wyeth Laboratory in Pennsylvania and by the Mérieux Institute in France, became commercially available in the United States in July 1982. By recombinant DNA technology, the Wistar Institute identified the complete nucleotide sequence for the gene responsible for the expression of the viral glycoprotein that induces neutralizing antibodies and protection against rabies. The complementary DNA gene has been cloned in a plasmid vector and expression of the gene attempted in a bacterium (*E. coli*), SV40 virus, papilloma virus and vaccinia virus. Only vaccinia-rabies glycoprotein recombinant was shown to produce rabies virus glycoprotein. The usefulness of this system for vaccine production is under investigation. The glycoprotein molecule has been analyzed and found to contain a portion that protrudes through the cell membrane, a transmembrane portion, and a cytoplasmic region. When cleaved, the outer portion is soluble and nonprotective. The transmembrane portion and part or all of the cytoplasmic domains are necessary to make the molecule immunogenic. These findings have an important bearing on development of a rabies sub-unit vaccine.

F32 AI 06733-01 P. A. Offit (Children's Hospital of Philadelphia): Human rotaviruses are responsible for severe episodes of diarrhea in very young children. Recently the "Wa" strain of human rotavirus was cultivated by others in primary cell cultures after 11 passages in "germ-free" piglets. Direct cultivation from fecal specimens now has been accomplished. Dr. Offit has used this new technology to study the growth characteristics of human rotaviruses in vitro and has described the structural polypeptides of purified virus particles. Under conditions of multiple-cycle growth in large vessels, a high concentration of tissue culture grade trypsin in the growth medium was required to obtain production of a high titer (10^7 plaque-forming units per milliliter) of infectious virus. Virions analyzed by electrophoresis showed five proteins with molecular weights ranging from 41,000 to 116,000 that were components of the inner virus shell; four other proteins were associated with the outer virus shell. The ability to grow the "Wa" human rotavirus in large quantities allows an analysis of the role of individual polypeptides in eliciting a protective immune response.

R01 AI 16102-04 M. J. Buchmeier (Scripps Clinic and Research Foundation): Applying the technology of monoclonal antibodies to the study of persistent viral infections, Dr. Buchmeier has surveyed brains of acute and persistently infected mice for expression of viral antigens in individual neurons. He found that while both the viral nucleoprotein (NP) and viral glycoprotein (GP) antigens are expressed during acute infection with lymphocytic choriomeningitis virus, the expression of viral GP diminishes to undetectable levels during the progress of a persistent infection. At the electron microscopic level he found by immuno-peroxidase labeling that viral NP is expressed in widely distributed neurons in the brains of mice infected for 6 or more months. The NP was associated with structures in the cytoplasm of infected neurons but the GP was undetectable. Such a selective expression of viral glycoprotein may provide an explanation by which virus infected cells escape immune recognition in vivo.

R01 AI 09484-14 M. B. A. Oldstone (Scripps Clinic and Research Foundation): Evidence in vivo that viruses can persist in differentiated cells and alter their function without imposing destruction has been revealed for the first time by Dr. Oldstone's staff. They noted that lymphocytic choriomeningitis virus (LCMV) displays a preferential affinity for the anterior lobe of the murine pituitary

gland. Further, this virus replicates in cells that make growth hormone but not in cells that make prolactin, thyroid stimulating hormone or adreno-corticotropic hormone. Replication of LCMV in cells that made growth hormone resulted in diminished synthesis of the hormone and a concomitant clinical picture of retarded growth and hypoglycemia in infected mice. These dramatic changes occurred without morphologic evidence of cell necrosis or inflammation in the anterior lobe of the pituitary. Consequently, during infection *in vivo*, a noncytopathic virus evidently turned off the "differentiation" or "Luxury" function of a cell, while not killing that cell. This in turn disrupted homeostasis and caused disease. Thus, a new mechanism by which virus causes disease was uncovered.

R01 AI 18449-01 W. T. Ruyechan (Uniformed Services University of the Health Sciences): Varicella-zoster virus (VZV), a herpesvirus is the putative agent of chickenpox and shingles. The virus is very difficult to grow in laboratory cultures, but Drs. Ruyechan and Hayes have succeeded in studying the intact viral DNA genome and cloned fragments. They have found the overall genomic structure to differ markedly from that of herpes simplex viruses, cytomegalovirus and Epstein-Barr virus, which also cause human disease. VZV DNA, however, does show some relationship to herpesviruses of veterinary importance, i.e., pseudorabies virus and equine herpesvirus types 1 and 3. Among 17 VZV strains, differences in DNAs have been noted and mapped. These differences appear to be the result of single insert/deletion events and are important because they occur as the result of interaction of the virus with its human host. Strains passed extensively in cell culture do not show such variability. Recently, these investigators also have made practical application of VZV DNA cloned fragments in developing a rapid technique for the laboratory diagnosis of chickenpox and shingles.

R01 AI 15134-05 G. T. Wertz (University of North Carolina, Chapel Hill): Dr. Wertz has demonstrated that it is possible to achieve replication of full length viral RNA and defective viral RNA outside of host cells. This *in vitro* replication is significant because it allows for study of each step of the replication process without complications introduced by the host cell. This work has shown that RNA replication of vesicular stomatitis virus (VSV) requires the synthesis of only the VSV nucleocapsid protein (N). It also demonstrated the assembly of newly synthesized VSV proteins with newly replicated RNA to form nucleocapsid structures *in vitro*. An understanding of mechanisms by which viruses replicate and express their genes will make it possible to identify factors that are crucial for viral replication that may be unique to the virus yet are different from cellular processes. These processes would be ideal targets for the development of anti-viral agents that could control viral infection without adversely affecting host function.

CLINICAL AND EPIDEMIOLOGICAL STUDIES BRANCH

The CESB serves as the Institute's focus for the development and processing of Investigational New Drug Applications, and maintains liaison with the NIH Human Research Review Panel and the NIAID-Clinical Research Subpanel. It provides and monitors a closed clinical facility for Institute volunteer studies, and conducts, promotes and supports the development and evaluation of procedures for the diagnosis, prevention and treatment of infectious diseases, particularly those procedures leading to improved patient care. The Epidemiology and Biometry (EB) Section conducts epidemiological research in infectious, allergic and immunological diseases, and analyzes data from various sources for trends in U.S. Vital Statistics in areas of interest to the Institute. The EB Section manages the Institute's program for mathematical and statistical studies, and provides biometrical consultation to other branches and laboratories.

Approximate Level of Support

<u>Activity</u>	<u>Number</u>	<u>Amount</u>
<u>Epidemiology, Antibiotic Trials, Rapid Viral Diagnosis, Nutrition, Volunteer Facility, Acquired Immunodeficiency Syndrome (AIDS)</u>		
Research Contracts	4	\$1,951,884
Research Grants	5	\$400,838
Cooperative Agreements	4	\$960,634
Interagency Agreements	1	\$10,000
Fellowships	1	<u>\$17,736</u>
Total	15	\$3,341,092
<u>Diarrheal Diseases</u>		
Research Contracts	1	\$455,145
Research Grants	30	\$2,901,260
Training Grants	1	\$34,992
Fellowships	1	<u>\$17,736</u>
Total	33	\$3,409,133
<u>Branch Total</u>		
Research Contracts	5	\$2,407,029
Research Grants	35	\$3,302,098
Cooperative Agreements	4	\$960,634
Interagency Agreements	1	\$10,000
Training Grants	1	\$34,992
Fellowships	2	<u>\$35,472</u>
Total	48	\$6,750,225

Enteric Diseases Program

The Enteric Disease Study Unit at the University of Texas in Houston (Dr. H. DuPont) concluded its contract and studies of diarrheal disease in day care centers. Sixty day care centers (DCC), randomly selected from 736 licensed child

care facilities in Harris County, were surveyed for the incidence of diarrhea by weekly telephone calls for two years. A total of 2,708 episodes of diarrhea occurred in children between the ages of 0-6 years, and 84 cases occurred among the teaching staff. The overall incidence was 0.9 cases per 100 person-weeks of observation in children and 0.3 among teachers. DCC characteristics which correlated with incidence among children were size of enrollment, presence of non-bowel-trained children and staff who both prepared food and cared for infants. In a separate study four non-randomly selected DCC were surveyed for outbreaks of diarrheal illness during which etiologic and transmission factors were investigated. Five teachers and 154 children submitted stool specimens during 13 outbreaks. Pathogens were detected in 43% of specimens from children with diarrhea and in 21% of specimens from asymptomatic children. Multiple pathogens were recovered during five outbreaks, and no pathogens were detected in two of the 13 outbreaks. Rotavirus and Giardia lamblia were the agents most often recovered regardless of health status.

Clostridium difficile was associated with five outbreaks of diarrhea occurring in three Houston DCC caring for children less than two years of age. The frequency of isolation of C. difficile and its toxin was determined in stool specimens from 65 children attending these centers. Twelve of 21 (57%) children who had diarrhea excreted C. difficile and its toxin, whereas only four of 44 (9%) who did not experience diarrhea yielded the organism and the toxin. Five of the 12 symptomatic children with C. difficile and its toxin had received prior antimicrobial therapy for upper respiratory tract infections. A study of environmental surface contamination with rotavirus was performed during three non-outbreak periods. Four of 25 (16%) samples collected from environmental surfaces and teachers' hands at a DCC were positive for rotavirus antigen using a fluorescence assay. The survival of two animal viruses, rotavirus SA-11 and poliovirus type 1, and of bacteriophage F2 on similar environmental surfaces in the laboratory were examined. Poliovirus type 1 and bacteriophage F2 were more resistant to drying than rotavirus SA-11 and could be recovered after a 90 minute exposure on a dry surface. Rotavirus SA-11 could be detected for 30 minutes. All three viruses survived longer when suspended in fecal material than in distilled water. These data suggest that several agents, including rotavirus, can remain viable on contaminated surfaces long enough to be transmitted to a susceptible child.

Investigators at Children's Hospital National Medical Center, Washington, D.C., have continued surveillance of infants and children entering the hospital with gastroenteritis. Data from these studies provide background for research-oriented epidemiologic projects, and the patients from such studies have yielded materials for virus growth, serotyping and "genetic finger-printing" methodologies. Rotaviruses and enteral adenoviruses, important causes of pediatric enteritis, have been cultivated recently. Eighty specimens which contained rotaviruses on the basis of EM and ELISA tests were inoculated into MA 104 cells. Sixty-two (78%) of these viruses grew to ELISA-positivity on at least two passages and 60 of these viruses grew sufficiently well to be detected by direct EM of unconcentrated cell culture fluids. These laboratory procedures were utilized during two nosocomial outbreaks of enteritis in a tertiary care nursery, where 22 of 102 infants had human rotavirus (HRV) infection. The time sequence and proximity of the infected infants in the nursery rooms had suggested baby-to-baby spread of a single HRV strain in each outbreak. However, polyacrylamide gel electrophoresis of virus permitted demonstration of four different HRV electropherotypes (strains) during the first outbreak and seven during the second. It was concluded that baby-to-baby spread of at least two different viruses apparently occurred in each outbreak period. By electrophoretic analysis, it was

demonstrated that babies in a tertiary care center can be infected with at least ten different HRV strains within two months, although any one child was infected with only one strain; that multiple rotavirus strains can spread independently and in parallel to different babies as part of a single outbreak; and that secondary spread of individual strains can be traced with considerable accuracy.

The Enteric Disease Study Center at the White Mountain Apache Indian Reservation in Arizona continues to make good progress. Several populations are being monitored for diarrheal illness and a number of special studies have been carried out during the past year. Preliminary analysis of the data confirms that the highest attack rate of diarrhea occurs in children under two years of age; the peak incidence of diarrhea on the reservation occurs in summer months; and the most common etiologic agents of summer diarrhea are Shigella sp. It has also been confirmed that the most frequent etiologic agent associated with diarrheal illness in the fall months is rotavirus. Campylobacter infections tend to cause diarrhea in approximately 10-15% of patients during the spring and summer months. During this year, a study was conducted on the use of three different types of oral rehydration solutions (ORS) in ambulatory patients. Solutions containing 90, 50 and 30 m.mol/L of Na⁺ and 2G/L of glucose were compared to a standard therapy (use of "Pedialyte") which contains 30 m.mol/L of Na⁺ and 5 G/L of glucose. No adverse effects were seen due to therapy in any of the groups. However, patients receiving ORS containing 90 and 50 m.mol/L of Na⁺ gained more weight two weeks after resolution of illness compared to other groups. Another special study initiated during this year included a double blind trial to evaluate the efficacy of erythromycin versus trimethoprin-sulfamethoxazole in treating Shigella and Campylobacter infections.

The CESB continued to coordinate the activities of the Cholera Panel of the U.S.-Japan Cooperative Medical Science Program. The 18th Joint Conference on Cholera Research was held November 28-December 1, 1982 in Kurishiki City, Japan.

Closed Volunteer Facility

The CESB supports a research contract with the University of Maryland to maintain the Center for Vaccine Development (CVD) where volunteers are studied before and after they are challenged with potentially transmissible infectious agents and candidate vaccines. From July 1, 1982, through June 30, 1983, 28 inpatient studies (365 volunteers) and six outpatient studies (200 volunteers) were carried out at the CVD. These studies were carried out under 11 active protocols approved by the institutional review boards of the University of Maryland and the National Institute of Allergy and Infectious Diseases. Included were Escherichia coli (3 studies), cholera (14 studies), viral diarrhea (1 study), Campylobacter (1 study), Shigella (3 studies), influenza (10 studies), oral typhoid vaccine (1 study) and phase one drug (1 study). Most of these studies are briefly summarized below.

Cholera challenge and rechallenge studies were carried out to determine the quality of immunity conferred by prior clinical infection with El Tor V. cholerae. Within the El Tor biotype prior clinical Inaba or Ogawa infection conferred significant protection against diarrhea upon rechallenge with either the homologous or heterologous serotype. However, the occurrence of two clinical breakthroughs in sequential El Tor cholera challenges is distinct from prior experience involving rechallenge studies with classical vibrios where protection was complete. Additionally, positive stool cultures were frequently found in

clinically-protected rechallenged El Tor veterans, whereas stool cultures were negative in volunteers rechallenged with classical cholera.

Three clinical studies were performed to evaluate the response in man to purified cholera enterotoxin given orally. The purpose of these studies was to identify a dose of cholera enterotoxin that causes diarrhea for use in challenge studies to evaluate pure antitoxic immunity and to select a safe dose of procholeragenoid for oral immunization. A 25 mcg dose of enterotoxin caused severe and unexpected purging (circa 22 liters). In subsequent feedings 0.5 mcg and 2.5 mcg doses caused no clinical effects. A 5.0 mcg dose caused moderate diarrheal illness in 4/5 volunteers. These results establish firmly for the first time in man that pure enterotoxin in low doses can induce cholera.

Based on the dose response studies with purified cholera enterotoxin, doses of 50 and 200 mcg of procholeragenoid were selected for immunization studies in man. No adverse clinical effects were observed in any of the volunteers at either dose. Three doses of combined procholeragenoid (50 mcg) and killed whole cell vaccine were given to a group of 20 volunteers who were then challenged with El Tor Inaba. All six controls and 11/15 (73%) of the vaccinees developed diarrhea. The difference in the occurrence of diarrhea between the two groups was not significant; vaccine efficacy was only 27%.

Two studies were carried out to evaluate the immunogenicity and efficacy of a combined B subunit and killed whole-cell oral cholera vaccine. Nineteen volunteers were given three doses of the combined vaccine. Approximately one month later 11 vaccinees and a group of seven unimmunized controls were challenged with El Tor Inaba. All seven controls (100%) and 4 of 11 of the vaccinees (36%) developed diarrhea. The difference between the two groups was significant ($p=0.01$); vaccine efficacy was 64%.

Dr. James Kaper in the CVD Bacterial Genetics Laboratory was able to delete the genes that encode for the production of enterotoxin from V. cholerae El Tor Inaba strain N16961. This genetically engineered strain, designated JBK70, was given to volunteers at doses of 10^6 , 10^8 and 10^{10} . Diarrhea was observed in 25% (1 of 4) of the group who received 10^6 , 40% (2 of 5) of the group who received 10^8 , and 60% (3 of 5) of the group who received 10^{10} . The development of diarrhea is presumably a consequence of colonization of the small intestine or the effect of yet another virulence factor, such as the newly discovered enterotoxin in JBK70. The local and systemic immune responses of these volunteers are pending. After one month a challenge study with the virulent parent of JBK70 was carried out to evaluate the protective effect of inoculation with JBK70. Only 1 of 10 JBK70 veterans (10%) developed diarrhea while 7 of 8 of the controls (87.5%) became ill, indicating that classic cholera enterotoxin is only one of several virulence factors in V. cholerae.

Clinical and laboratory studies were carried out to develop vaccines that can stimulate anti-adhesion (anti-pili) immunity against diarrhea caused by enterotoxigenic E. coli (ETEC). In collaboration with the Walter Reed Army Institute of Research, twelve volunteers received eight oral doses of a purified CFA/II pili vaccine given twice a week for four weeks. One month later 6 of the 9 controls and 3 of 8 vaccinees developed diarrhea in response to challenge with virulent ETEC. These poor results once again point to the probable superiority of oral living vaccines over killed vaccines for prevention of enteric infections.

Three studies were carried out to evaluate the immunogenicity and efficacy of a newly developed bivalent *S. typhi/S. sonnei* live attenuated oral vaccine. Three doses of the bivalent vaccine were given orally to 16 volunteers. One month later 6 of 8 controls (75%) and 2 of 10 vaccinees (20%) developed illness in response to challenge with the virulent *S. sonnei* strain 53G. This promising dysentery vaccine will be studied further.

During the autumn of 1982, the CVD in collaboration with the LID, NIAID conducted a large influenza vaccine study at the College Park campus of the University of Maryland. Approximately 400 students were screened for seroeligibility, and 125 had histories and physicals; 115 student volunteers were finally administered either A/Washington/80 (H_3N_2) or A/California/78 (H_1N_1) cold-adapted (ca) reassortant virus vaccine intranasally or inactivated subvirion parenteral vaccine. A/Washington/879/80 (H_3N_2) and A/California/78 (H_1N_1) wild type virus challenges were administered to vaccinees and seronegative controls. Evaluation of illness responses, antibody responses and viral titers are in progress.

A series of seven studies were carried out to evaluate the level of attenuation and immunogenicity of a newly developed reassortant influenza A virus vaccine containing the surface antigen genes of human influenza A viruses and the six "internal" genes derived from an avian influenza virus, A/Mallard/78 (H_1N_1). The results show that these new vaccine candidates are suitably attenuated in adults. Infectivity and immunological tests are presently in progress.

One study was conducted in a group of 11 volunteers who ingested an inoculum of Norwalk agent. Six of 11 volunteers developed vomiting and/or diarrhea during this study. Isolation of virus from stool and immunological assays are in progress.

Collaborative Clinical Trials of Antibiotic Therapy

The contract with the University of Alabama to study improved therapy of cryptococcal meningitis and other fungal diseases continues to provide meaningful clinical and laboratory data. In Study #1, 193 patients with cryptococcal meningitis have been enrolled. Among the 43 protocol adherent patients who received Regimen A (four weeks of combined amphotericin B and 5-flourocytosine) nine relapses have occurred. By comparison, among 44 protocol adherent patients who received Regimen B (six weeks of combined drug therapy) six relapses have occurred. Enrollment into this trial was concluded in July, 1983. A statistical evaluation of the data is in progress to determine the probability of achieving a significant difference between the two regimens.

Study #2 entitled "Phase II Trial of Ketoconazole in Five Different Systemic Fungal Infections" has been completed by the contractor. Toxicity and clinical response were studied in 52 patients. Cure or marked improvement occurred in 27 patients (52%), whereas failure of the primary course was seen in 14 (27%), and relapse after ketoconazole was discontinued was noted in 11 (21%). In 35 patients (67%) no evidence of toxicity was observed. Nausea, anorexia, or vomiting occurred in 21%. The results from this trial indicate that ketoconazole, in the dosage regimens used (100-600 mg per day), was more effective in patients with histoplasmosis and non-meningeal cryptococciosis than in patients with blastomycosis and non-meningeal coccidioidomycosis, and least effective in patients with sporotrichosis.

Enrollment into Study #3A was completed in December, 1982. This trial compared the efficacy and toxicity of two different dosage regimens of oral ketoconazole in randomly assigned patients with either blastomycosis or histoplasmosis. Eighty-three patients with blastomycosis were enrolled. Forty-one patients were randomized into Regimen A (400 mg/day) and 42 were randomized into Regimen B (800 mg/day). Currently, among 41 patients in Regimen A, 24 have completed therapy and are considered cured, eight patients remain on therapy, four patients failed the primary course of therapy, one patient relapsed, and four patients were judged protocol in-adherent. Among the 42 patients in Regimen B, 21 have completed therapy and are considered cured, 14 remain on therapy, none have failed, one relapsed and six were judged protocol in-adherent. There is preliminary evidence to show that ketoconazole at both low and high doses is an effective alternative to amphotericin B therapy for non-life-threatening and non-meningeal blastomycosis. Whereas low dose ketoconazole is better tolerated than high dose ketoconazole, high dose ketoconazole may be more effective.

In the prospective randomized trial of two different dosage regimens of ketoconazole for the therapy of histoplasmosis, 55 patients have been enrolled. Thirty two have been randomized into Regimen A and 23 into Regimen B. In the Regimen A group of 32 patients, 15 patients have completed therapy and are considered cured, 10 remain on therapy, one failed the primary treatment course, one relapsed and five were judged protocol in-adherent. Among the 23 Regimen B patients, 10 have completed therapy and are considered cured, six remain on therapy, one relapsed and six were judged protocol in-adherent. It appears that ketoconazole at both low and high doses is an effective alternative to amphotericin B therapy for all non-meningeal forms of histoplasmosis. However, the low dose regimen of ketoconazole is better tolerated.

Twenty-nine patients have been enrolled into Study #3C entitled "Pharmacokinetic and Toxicity of Various Doses of Ketoconazole in Coccidioidal Meningitis." Very high doses of ketoconazole were used (800-2,000 mg/day). Six patients have completed the protocol and remain on therapy; 17 remain on the protocol; four were protocol in-adherent due to secondary toxicity; one refused further participation; and one died (not drug related) six days after entering the study. Adverse effects include: nausea and/or vomiting, 27%; gynecomastia, 17%; alopecia, 6.8%; and one patient experienced decreased libido. Concentrations of ketoconazole in CSF and serum are being determined by Dr. David Stevens at the Santa Clara Valley Medical Center, California. When drug level data are available, this information will be correlated with clinical response and toxic side-effects.

During the past year, a trial of single-dose therapy with sulfamethoxizole-trimethoprim in women with dysuria has been initiated by Dr. Walter Stamm and co-workers at the University of Washington. The purpose of this study is to obtain definitive answers concerning the optimal management of adult women who present with dysuria and no evidence of vaginal infection. Single-dose therapy will be compared with one week of sulfamethoxizole-trimethoprim therapy in unselected women who present to the University of Washington's Family Medical Center with lower urinary tract symptoms. The trial will be prospective, randomized, double blind and designed to compare efficacy and cost-effectiveness. Dr. Stamm and his colleagues are proven investigators in the field of sexually transmitted disease, urinary tract infection and the dysuria-frequency (acute urethral) syndrome. It is anticipated the trial will require 30 months to complete.

Nutrition, Infection and Immunity

The goal of this program is to promote research on the interaction of malnutrition, infection and immunity. Specifically, our interests focus on the modulating effect of specific nutrients on immune function and microbial virulence, on mechanisms of food allergies and immune response to ingested antigens, and on the interaction of nutrition and infection in the tropical environment and in American hospitals.

In FY 1982 the program consisted of 21 grants, subprojects of one contract, one Research Career Development Award, one New Investigator Award, one Postdoctoral Fellowship, one training grant, one interagency agreement, and one intramural project on mechanisms of Food Allergy. The extramural program was monitored by at least four project officers in the IAID and MID Programs, in addition to the CESB.

Rapid Viral Diagnosis

Dr. Schmidt (State of California, Department of Health Services) has developed a number of monoclonal antibodies which she is using to develop rapid diagnostic tests of early infection. One of her monoclonal antibodies to varicella zoster virus had broad immunofluorescent activity and reacted with all of the clinical isolates tested in an anti-complement immunofluorescence (ACIF) test. The ACIF test is better than the widely used fluorescent antibody-to-membrane antigen test because it is more sensitive, can be used to examine sera at low dilutions (1:2-1:4), and is more economical in terms of antigen and personnel time.

For group B coxsackieviruses, an enzyme immunoassay (EIA) using a fluorogenic substrate has shown 100% agreement with independent neutralization tests of more than 100 field strains of coxsackievirus. This method is being explored for direct identification of virus in stool specimens. An EIA for the detection of IgM antibody to group B coxsackieviruses was used in a study of sera from 20 cases of juvenile-onset diabetes and 20 matched controls. No IgM antibody to coxsackievirus types 1-6 was present in any of the cases.

A direct immunofluorescence (DIF) staining test which involves the use of three monoclonal antibody conjugates (H_1 and H_3 of influenza A virus, and group reactive influenza B virus) has been developed. Identification of specimens with DIF staining correlated with the hemagglutination inhibition test results, but was less cumbersome and more sensitive, especially for the H_1 influenza viruses which produce little hemagglutinin. Monoclonal antibodies to human IgM were produced in mice. Using these reagents, a new EIA detected IgM antibody to rubella virus in congenitally infected infants in whom it was not possible to demonstrate rubella IgM antibody by immunofluorescence staining. An immunofluorescence assay for IgM antibody to Ureaplasma urealyticum detected such antibody in 17 of 713 sera from newborns and infants with severe, congenital neurological disorders or with respiratory infections occurring after birth. The use of monoclonal antibodies to human IgM should permit the development and standardization of reliable viral IgM antibody assays which can be used for early diagnosis of congenital and postnatal bacterial and viral infections.

Dr. Richman and his collaborators (University of California, San Diego) have continued to detect virion proteins using an immunofiltration assay. They are extending their studies to increase the assay's speed, sensitivity and practical applicability. In addition, they are using cloned DNA probes to detect virion

nucleic acid by hybridization. They have developed an assay which uses nucleic acid hybridization with a 32 P-labeled DNA probe prepared from a fragment of herpes simplex virus (HSV) DNA cloned in a plasmid vector. The assay can detect 5×10^4 plaque-forming units of cell-free HSV and as few as four virus-infected cells. It has a sensitivity of 78% and a specificity of 100% compared to culture for the detection of HSV in swab specimens from genital lesions. Specimens from herpes zoster lesions are uniformly negative. Type specific HSV-1 and HSV-2 probes are also available. They are attempting to convert the assay from one using a 32 P-labeled DNA probe to one using a biotinylated probe which utilizes a colorimetric enzyme detection system. Such an assay would be more rapid, be readily performable by any laboratory, and use reagents that have an indefinite shelf life.

Dr. Yolken (Johns Hopkins) has been working on methods to increase the practicality of EIA techniques by using suitable enzyme substrate systems read visually. Beta-lactamases were found to give clear cut visual readings; they are highly active, widely available in nature, contain active amino groups removed from the active site of the enzyme which allows them to be covalently labelled with markers such as biotin, and have a large number of substrates available for the measurement of beta-lactamase reactions. Using a biotin enhanced EIA, as little as 0.4 nanograms of H. influenzae type b PRP could be detected. When applied to the detection of viral agents, the system could detect between 1 and 10 TCID₅₀ enteric type adenovirus, and rotavirus antigen in 36 of 37 clinical specimens previously shown to contain rotavirus. The results were easily read and could be recorded by the use of a standard office photocopying machine, which allows for the maintenance of a permanent record without the need for expensive instrumentation.

An alternative to preparing and using monoclonal antibodies as reagents in EIAs is the use of antibodies from an immunized chicken. Under appropriate conditions the chicken will produce serum antibody which is passed efficiently and reproducibly to the yolk sac. Chicken yolk sac antibodies (IgY class) to influenza virus, adenovirus and rotavirus could be used either as solid phase antibodies or as the second antibodies in sandwich systems which could be labelled directly with biotin or enzyme. The sensitivity of the EIAs using yolk-derived antibodies compared favorably to ones using antibodies from standard laboratory animals and to ones using monoclonal antibodies. An egg can yield up to 20 mls of yolk containing 300 mgm IgY, and chickens can lay an egg a day for an extended period of time thus promising a large scale source of antibodies for use as reagents in immunoassays.

Acquired Immunodeficiency Syndrome (AIDS)

The National Cancer Institute issued a request for cooperative agreement applications (RFA) on August 13, 1983 entitled, "Studies of Acquired Immunodeficiency Syndrome (Kaposi's Sarcoma and Opportunistic Infections)." Over 40 investigators responded to the RFA. Of these applications, the NCI funded nine, while NIAID funded four which cost NIAID about 1,000,000 dollars in FY '83. Dr. Robert Edelman was named project officer of these four cooperative agreements, located at the Albert Einstein College of Medicine, Bronx, N.Y. (Dr. Arye Rubinstein), U.C.L.A. (Dr. John Fahey), St. Vincent's Hospital, N.Y.C. (Dr. Pearl Ma) and St. Jude's Hospital, Memphis, (Dr. Walter Hughes). Dr. Rubinstein's proposal focuses on the epidemiology and immunology of pediatric AIDS and AIDS in I.V. drug addicts; Dr. Fahey is investigating the immunology and therapy of AIDS; Dr. Ma is

studying cryptosporidiosis; Dr. Hughes is attempting to develop better chemotherapy of Pneumocystic carinii pneumonia. A joint NCI-NIAID cooperative agreement working group, consisting of NIH staff and newly funded investigators, met May 6, 1983 to exchange research information, coordinate efforts, and to begin to develop working definitions of adult AIDS, AIDS related complex (prodrome), pediatric AIDS, and staging for Kaposi's sarcoma.

A NIH Research Workshop on The Epidemiology of AIDS was held at the Holiday Inn Crowne Plaza, Rockville, Maryland, September 12-13, 1983. The workshop, sponsored by NIAID, NCI, and NHLBI, developed recommendations for research on the epidemiology and natural history of AIDS for possible implementation by NIH. The workshop also provided an opportunity to discuss epidemiology study design and the obstacles impeding valid epidemiological studies of AIDS. Dr. Edelman served as scientific coordinator of the workshop. Additional branch AIDS research activity is summarized under the Epidemiology and Biometry Section of this report.

Epidemiology and Biometry Section

The Epidemiology and Biometry Section (EBS) has continued to pursue epidemiologic research in infectious and immunologic diseases and biometric research and consultation in these areas. In addition to Dr. Kaslow and Dr. Blackwelder, Dr. Saah has been appointed to the unit's staff. Other part time and temporary professionals have been engaged in selected epidemiologic activities. Our second Epidemiology Training Associate, Dr. Anne Bailowitz, has finished her MPH training and joined the EBS office.

Infectious Diseases

The unit has been heavily involved in planning a three-year prospective effort to study the natural history of AIDS in homosexual men. Subjects will be given a questionnaire for the purpose of correlating any current biologic abnormalities or manifestations of disease with sexual practices and other exposures. Additionally, serum, urine, feces, and other body fluids will be collected and banked for future evaluations, e.g., when a marker for the disease becomes available, and for virus isolation. The attempt is to collect, in a single large study, information that encompasses the time period from before infection to the development of disease in a sufficient number of high risk individuals to permit meaningful epidemiologic statements. The EBS, in conjunction with Dr. David Henderson, Infection Control Officer, has designed a prospective study of Clinical Center employees and their exposure to patients with AIDS. Follow-up for three to four years will consist of periodic questionnaires and, if appropriate, detailed immunological studies. Dr. Saah was among several NIAID staff who consulted with investigators in Haiti on possible risk factors for AIDS in Haitians.

The discovery of a tick-borne spirochete as the putative cause of Lyme disease led to a successful team effort to isolate the organism from patients in New York and publication of these findings. Analysis of data from a 1982 serosurvey on Fire Island is nearing completion, as are the efforts at serologic confirmation of reported Long Island cases. In conjunction with Dr. J. Bruce McClain (WRAMC), an ELISA has been developed that will greatly facilitate seroepidemiologic studies. Joint activities with the State of Wisconsin have generated considerable descriptive data on occurrence of the disease there and on the ecologic factors involved in its transmission.

Work has continued with Dr. LaMontagne (DAB) and others on influenza morbidity and mortality at the Jewish Home and Hospital for the Aged in New York City. The past year's effort involved continuation of a prospective, non-randomized clinical trial of influenza vaccine with virologic and serologic studies. Drs. Blackwelder and LaMontagne also completed analysis of the 1978 Russian influenza vaccine trial. Drs. Kaslow and Blackwelder have begun exploring the NCHS multiple cause mortality statistics for additional insights on deaths from pneumonia and influenza.

Considerable time was spent by unit staff along with Dr. Edelman to develop a major collaboration with NICHD on the role of genitourinary infections and prematurity. The work, to be performed under contracts at several centers, will examine the risk factors, especially the microbial flora of the female lower reproductive tract. If the circumstances are favorable, the study design calls for emphasis on antimicrobial prophylaxis in a randomized trial. Addressing this same issue, Dr. Kaslow has made two trips to India during the past year to consult on a similar research project by Indian investigators. Progress has also been made toward assembling data from the Collaborative Perinatal Project to re-examine the role of urinary tract infection and prematurity.

Through his connection with the Egypt-Israel project on vector-borne diseases, Dr. Fred Feinsod and his Egyptian colleagues have important clues to the likely path of introduction of Rift Valley fever into Egypt in 1977. This introduction has been an enigma, and its elucidation would assist Rift Valley fever control efforts in the future.

Immunologic Diseases

The Collaborative Perinatal Project records of approximately 1,200 children with asthma have been abstracted under Dr. Saah's direction. Analysis of patterns of disease and of exposure variables in their mothers is planned. Drs. Bailowitz and Koslowe assisted the Maryland State Health Department in investigating an outbreak of Kawasaki syndrome in Baltimore and outlying Maryland counties during the spring. They collected data to test the hypothesis that children with the disease more often come from homes where carpets have been cleaned within a few weeks prior to onset. Preliminary analysis has suggested that such an association is unlikely.

Both environmental exposure studies and early immunogenetic studies on systemic lupus erythematosus (SLE) patients are nearing completion. Data analysis is well underway from a case-control study of SLE patients in collaboration with Dr. Marc Hochberg, Johns Hopkins University. Interesting data on the reproductive histories and on history of allergy were obtained.

Dr. Blackwelder continued work with Dr. Robert Elston of Louisiana State University on the use of sibship data to detect genetic linkage between a disease susceptibility locus and a polymorphic marker, with particular reference to the HLA system. They have been comparing various methods for analysis of sib-pair data.

Biometric Consultation and Research

Dr. William Blackwelder served as consultant to NIAID project officers and as a member of data monitoring committees for collaborative clinical trials on: 1) comparison of two durations of therapy with amphotericin B and 5-fluorocytosine

in cryptococcal meningitis, 2) efficacy of adenine arabinoside monophosphate alone and combined with human leukocyte interferon in chronic hepatitis B infection, 3) efficacy of antiviral substances in herpes encephalitis and neonatal herpes infections, and 4) efficacy of interferon therapy for pediatric papillomatosis.

In another area of biometric research, he investigated properties of common statistical tests for comparing binomial proportions when the proportions are small.

Other Activities

The Investigational New Drug Applications (INDAs) for the Microbiology and Infectious Diseases Program are filed and maintained by the CESB. Currently 30 active INDAs are on file with the National Center for Drugs and Biologics, Food and Drug Administration. Five INDAs are for drugs and 25 are for biologics. During the past year new INDAs have been filed for human leukocyte interferon for the treatment of laryngeal papillomas, B-subunit of cholera toxin, two oral cholera vaccines, gamma interferon for the treatment of AIDS patients, an oral E. coli CFA/II pili vaccine and an oral typhoid-Shigella sonnei vaccine. The gamma interferon IND was filed for an intramural protocol. The E. coli CFA/II pili vaccine and the typhoid-Shigella sonnei vaccine were prepared by the Walter Reed Army Institute of Research, and the NIAID is collaborating with them by supporting the clinical trials.

The branch staff served as Institute representatives on many Trans-NIH committees: Dr. Horton--Digestive Diseases, Diabetes, and Cystic Fibrosis; Dr. Kaslow--Epidemiology, Diabetes, USPHS Reye Syndrome Task Force; Dr. Edelman--Nutrition, Clinical Trials, National Digestive Diseases Advisory Board (ex-officio), selection committee exempting NIH clinical trials from OMB regulations, Institutional Review Boards of the Walter Reed Army Institute of Research and the NCI-Frederick Cancer Research Facility, Clinical Associate Professor of Medicine and preceptor in Internal Medicine, Uniformed Services University of the Health Sciences.

DEVELOPMENT AND APPLICATIONS BRANCH

The primary objective of the Development and Applications Branch is the translation of new information derived from basic research into methodologies appropriate for the control or prevention of infectious diseases. Individual program areas have been designated as high priority for extramural support by the Institute. Applied research activities include identification of important infectious disease problems with potential for control or prevention through immunization or utilization of antivirals or hyperimmune sera, development of appropriate vaccines, antivirals and other control measures, development of animal models and design and support of appropriate clinical trials for evaluation of control measures. In addition, basic research relevant to the individual programs is supported by the Branch.

Approximate Level of Support

<u>Activity</u>	<u>Number</u>	<u>Amount</u>
101,748,828 834,155,811	211	\$ 1,514,234
Vaccine Evaluation Units	5	\$ 1,514,234
181,811 181,811	1	
Research Contracts	5	\$ 2,204,366
144,381,852	25	3,347,314
	Subtotal	\$ 5,569,416
Influenza	4	\$ 1,514,234

<u>Activity</u>	<u>Number</u>	<u>Amount</u>
Research Contracts	5	\$ 2,204,366
Research Grants	25	3,347,314
Fellowships	1	17,736
	Subtotal	\$ 5,569,416
Respiratory Diseases	31	\$ 5,569,416
Research Contracts	0	0
Research Grants	0	0
Fellowships	1	17,736
	Subtotal	\$ 17,736
Hepatitis	9	\$ 722,997
Research Contracts	3	\$ 1,530,651
Research Grants	11	1,388,450
Interagency Agreements	2	228,172
Career Awards	1	55,707
Fellowships	2	18,468
	Subtotal	\$ 3,221,448

<u>Activity</u>	<u>Number</u>	<u>Amount</u>
Antiviral Substances	8	\$ 2,517,577
Research Contracts	11	\$ 2,517,577
Research Grants	30	3,091,255
Career Awards	1	41,472
Fellowships	1	660
	Subtotal	\$ 5,650,964

Bacterial Vaccines

Research Contracts	7	\$ 1,256,776
Research Grants	37	3,508,319
Career Awards	2	87,737
Fellowships	2	44,496
Subtotal	<u>48</u>	<u>\$ 5,650,964</u>

Viral Vaccines

Research Contracts	2	\$ 412,106
Research Grants	5	463,744
Subtotal	<u>7</u>	<u>\$ 875,850</u>

Branch Summary

Research Contracts	33	\$ 9,435,710
Research Grants	115	18,221,453
Interagency Agreements	2	228,172
Career Awards	4	184,916
Fellowships	7	990,096
Totals	<u>161</u>	<u>\$28,169,347</u>

Program Summary

Activities of the Development and Applications Branch include comprehensive research programs on influenza and other viral respiratory diseases, viral hepatitis, viral vaccines, bacterial vaccines and antiviral substances. Because these programs for development of control measures are designated as high priority by the Institute, funding is provided utilizing all mechanisms available to staff. The DAB carries out many of its objectives through directed research and is responsible for the major portion of the contract activities within the Microbiology and Infectious Diseases Program.

It has been noted in past annual reports that staff has been concerned about reduction of contract funds and the DAB's ability to perform its mission satisfactorily. Although DAB contract expenditures increased in FY83, much of the increase reflects the innovative funding techniques used by the Contract Management Branch and 3 new clinical trial awards with exceptionally large budgets. The drop from 44 contracts in FY82 to 30 in FY83 is more indicative of the further erosion of contract supported research. This situation will certainly reduce the ability to obtain "payoffs" from promising leads developed in basic research activities.

The winter of 1982-1983 was not a particularly devastating influenza season, but H3N2, H1N1, and influenza B viruses all produced outbreaks of varying severity. In addition to the effectiveness of rimantadine in preventing influenza infections (shown in 1981-1982), another drug, ribavirin, was shown to be effective for the treatment of severe influenza infections when administered by aerosol. Although not directly sponsored by the NIAID, studies showed this drug to be effective in treating clinically severe respiratory syncytial virus infections in infants. These observations need to be amplified and confirmed in the next year. Attenuated influenza vaccines continue to show great promise, with data on the cold adapted recombinants suggesting safety and efficacy. Basic research is

providing many answers on the molecular nature of surface antigens of the virus and their interactions with host cells.

The effectiveness of hepatitis B vaccines in preventing infections in most normal "at risk" adult populations has been established. Immuno-compromised groups may not receive the full benefits of the vaccine. The trial conducted in persons undergoing hemodialysis indicated that additional information is needed on vaccine dose, timing of vaccination, and nature of immune response before recommendations for usage can be made. Because of the expense of the licensed HBV vaccine, alternate vaccine antigen sources are required, an area of intense investigation. The DAB is prepared to undertake phase 1 and 2 testing of candidate preparations that may be developed. Animal models (woodchuck, ground squirrel, and Pekin duck) for hepatitis B infections are being vigorously explored by government scientists, contractors, and grantees. There is promise of an attenuated hepatitis A vaccine in the next 1-2 years, and small-scale volunteer testing may be initiated during FY84.

As information from basic virologic research becomes available, antiviral drug development will be targeted at inhibition of specific viral functions. Several new drugs specific for herpes-induced thymidine-kinase are now undergoing pharmacokinetic and toxicologic studies in animal models for assessment of potential usefulness in man. In addition to the various protocols for evaluation of adenine arabinoside, acyclovir and interferon for treatment of herpes virus infections in man, a new study has been undertaken for the evaluation of interferon for treatment of laryngeal papilloma in children. Also anticipated for this fiscal year is a controlled trial of treatment with interferon of Condyloma infections - an extremely common disease. The program continues to serve as a WHO Collaborating Center on Interferon Reference and Research. Human gamma interferon and a cloned leukocyte preparation were added to the available reference agents.

Collaborative efforts by the bacterial vaccines program have shown that various patient populations do respond to pneumococcal vaccine, but immunosuppressive treatment for their underlying illness can severely hamper that response. Pneumococcal vaccine may also protect against otitis media if the vaccine recipient develops type specific antibody after immunization. However, the lack of immunogenicity in infants under 2 years of age indicates that additional research is required to enhance the immunogenicity of the polysaccharide vaccine. An approach to enhanced immunogenicity employing conjugation of polysaccharide to diphtheria toxoid appears successful for *H. influenzae* antigen. Test preparations appear to be highly immunogenic in one-year olds, and plans are underway to initiate an efficacy trial of a PRP-toxoid conjugated vaccine this fiscal year.

The development of a new non-reactogenic *B. pertussis* vaccine has been of interest to the DAB for several years. A contract award for preparing an acellular vaccine will be awarded this year. In addition, it is hoped that clinical trials can be started that will use DTP that contains the "P" component developed by the Japanese. This vaccine is made by a U.S. manufacturer, utilizing bulk pertussis antigen purchased from the Japanese. The initial trials will be designed to measure reactogenicity and antigenicity of the "P" component. Efficacy trials of a new pertussis vaccine will most likely have to be performed outside of the U.S.

To date 188 children and 25 adults have been vaccinated with an attenuated varicella vaccine to determine its value in protecting the immunosuppressed against varicella infections. All vaccinees have responded immunologically, and though documented exposures in 17 vaccinees occurred, only 3 mild cases of chickenpox have occurred.

During the past year the vaccine evaluation units have tested candidate vaccines in adults and children; they have also evaluated some antiviral compounds such as amantadine, rimantadine, and interferon for safety and effectiveness.

Research Highlights

Influenza Program

Two antigenic types of influenza A viruses, H1N1 and H3N2, continue to circulate and produce epidemics in the human population. The epidemic of influenza which occurred last winter was not particularly severe. Three viruses, A/Philippines-like (H3N2), A/India-like (H1N1) and B/Singapore-like were commonly identified and all produced outbreaks of varying severity. The continued circulation of two distinct type A viruses remains a perplexing phenomenon. Whether this pattern will persist or what its duration will be are unknown. This illustrates once again the extent of our ignorance of the epidemiology of these viruses.

The year was marked by several important developments in influenza research. Rapid progress continues to be made in efforts to characterize influenza viruses at the molecular level. The sequence of the neuraminidase genes of type A and B viruses was obtained. Work by Lamb and Choppin revealed that the neuraminidase gene of type B viruses codes for two glycoproteins. Only one, the neuraminidase protein has been found in the virus particle. The second protein, about 100 amino acids long has been found in infected cells. A three-dimensional model of the neuraminidase was obtained based on crystallographic data. Work with the hemagglutinin has revealed that this protein undergoes a conformational change at pH 5.0. This conformational change exposes the hydrophobic amino terminus of HA2 and is required for the fusion of viral and cell membranes. Much has also been learned about the biochemical properties of the hemagglutinin. This work, done in several laboratories, has employed the use of recombinant DNA technology. In this way isolated genes can be introduced into cells and their behavior investigated without the interference of other viral proteins. Hemagglutinin genes in which specific regions of the gene have been deleted were studied using this approach. Hemagglutinin genes which lacked the signal sequence were found to produce incomplete hemagglutinin proteins which accumulated inside the cell, thereby, confirming the requirement of this sequence of amino acids in the transport of the viral protein across the cell membrane. Other work has revealed that the hemagglutinin has a specific tropism within the cell to the apical surface of the cell and that hemagglutinin genes constructed without the region which codes for the hydrophobic anchor of the molecule were released into the medium from infected cells. The hemagglutinin has not been the only success story. The careful analysis of the biochemical events of viral RNA replication continues. At least one biochemical function has been assigned to each of the three viral polymerase proteins. This promising work not only increases our basic knowledge of the molecular mechanism of nucleic acid replication but also points to a promising area for antiviral development.

The year was also marked with important advances in the development of antivirals for use in the prevention or treatment of influenza infections. The effectiveness of rimantadine was clearly demonstrated through clinical trials supported by the NIAID. This drug is as effective as amantadine and did not produce any of the side-reactions that have been related to amantadine use. A third drug, ribavirin, was also shown to be effective in the treatment of influenza A and influenza B infections when administered in a small particle aerosol. This is a promising first step in the development of new methods of drug delivery that may be of use in the treatment of severe respiratory infections.

Vaccine development remained the most important priority during the last year and important gains were made towards the goal of a safe, attenuated, stable, non-reactogenic and immunogenic influenza A vaccine. The ca vaccines developed through a contract with The University of Michigan continue to be promising vaccines. Clinical trials for efficacy were performed at three locations during the year, and preliminary indications are that the vaccines were effective and safe. Studies on the antigenicity and safety of bivalent type A vaccines were also performed with encouraging results. A comparison of inactivated and ca attenuated type A vaccines was also performed. The results of this study indicate that the ca vaccine has a marked effect on viral shedding patterns, while the inactivated vaccine did not. Research towards this goal is progressing rapidly. A new attenuated influenza type A vaccine candidate has been developed by NIAID scientists. This vaccine uses as the donor of attenuation an avian virus - A/Mallard/New York/6750/78 (H3N2). This donor can be used to rapidly and reproducibly transfer the attenuated phenotype to wild-type strains through genetic reassortment. Studies in animals and preliminary studies in man suggest that this vaccine offers promise. Clinical trials with the avian reassortant vaccines will continue. Progress with the type B ca vaccines has been slower due to the inherent variability in antigenicity and virulence of natural B isolates. In addition, there is less information on the genetic properties of B viruses.

Studies of the epidemiology of influenza viruses in man and animals have been an important priority of the influenza program. The community-based epidemiologic studies of the Influenza Research Center are the most important component of this effort. It is now clear that reinfection with influenza viruses can occur during the same season. Influenza viruses also disappear from the community during the summer months suggesting that each new epidemic is imported into the community each year. In addition, they have shown that patterns of virus isolation may represent an important harbinger of future epidemics. The role of animal myxoviruses and of the animal ecosystem as the source of new variants is still under active investigation. The asymptomatic carriage typical of influenza virus infections in migratory waterfowl is thought to be a likely source of new variants. Recent observations indicate that a virus, indistinguishable from typical swine influenza virus, was recovered from turkeys and was capable of causing disease in mammals.

Viral Respiratory Diseases Program

Progress in developing effective vaccines for respiratory syncytial virus (RSV) and parainfluenza has been painfully slow. Dr. Richard Compans, University of Alabama, has been attempting to isolate and purify surface glycoproteins of parainfluenza virus 3 for evaluation as a subunit vaccine. Efforts have been

severely hampered by the inability to produce adequately purified virus preparations. Methods previously used for other parainfluenza viruses (i.e., SV-5, Sendai) have been unsuccessful for this human pathogen.

Efforts at developing an effective immunogen for RSV are limited to the NIAID intramural program. Further evaluations of temperature sensitive mutants have been abandoned and approaches to live vaccine construction utilizing recombinant DNA technology are in progress. Approaches to identifying viral antigens important in stimulating host immunity are underway at the Laboratory of Molecular Virology and Immunology, Georgetown University. A series of monoclonal antibodies has been produced that is being used to characterize viral components. A recent publication by Investigators at Rochester indicates that a monoclonal antibody has been developed that is specific for the fusion (F) glycoprotein of RSV.

Detailed studies of the molecular virology of RSV will be undertaken by two new grantees, Drs. Gail Wertz and Peter Collins, University of North Carolina. Their innovative and modern approaches to the study of this difficult virus should provide significant advances in the forthcoming years.

Viral Hepatitis Program

The magnitude of the Public Health problems resulting from the hepatitis viruses (A, B, non-A non-B) has been well documented in previous Annual Reports. The goal of the Hepatitis Program is to explore and develop control measures for these viral infections. Type B viral hepatitis remains an area of intense research activity because of its more serious clinical manifestations and long-term sequelae.

The HBV vaccine efficacy trial in "at risk" staff and patients of hemodialysis centers has been completed. As reported last year, the vaccine (20 ug/dose) was highly effective in preventing infection in staff members. However, detailed analysis of the patient data (40 ug/dose) indicates that the vaccine was not highly efficacious in this immunocompromised population. The observed attack rates of 4.8% and 5.0% in the placebo group and vaccine group, respectively, after 18 months of follow-up were not significantly different. However, given the numbers in this trial and the observed attack rates, it would not be possible to show efficacy of a vaccine that is less than 50% effective. The response rate and duration of antibody following vaccination were significantly less than that in normal "at risk" populations.

Evaluation of HBV vaccine (prepared by NIAID) for the prevention of neonatal HBV is well underway in the Peoples Republic of China. Babies born of HBV chronic carrier mothers are immunized at birth, 1 month, and 6 months (16 ug/dose). More than 200 babies have been randomized into the trial thus far, but it is too early for determination of results. Thus far, the vaccine has been shown to be safe and non-reactogenic.

Because vaccine antigen is isolated and purified from plasma of HBV chronically infected persons (many of which are at risk for AIDS), this expensive biologic is under-utilized. Alternate sources of vaccine antigen are being sought by industry and academic institutions. Methodologies employed include recombinant-DNA techniques and synthesis of immunogenic polypeptides. Synthetic HBV polypeptides are being tested in chimpanzees through collaborative efforts of Dr. Richard Lerner, Dr. John Gerin, and Dr. Robert Purcell. Results to date have been mixed and much more research needs to be conducted. A proposal from Dr. Blaine

Hollinger, Baylor College of Medicine, has received an excellent review and the Institute should fund his R01 for the evaluation of a synthetic polypeptide vaccine in chimpanzees in FY84.

An experimental facility for woodchucks and their indigenous hepatitis virus (WHV) was established at Cornell University. A WHV vaccine similar to human HBV vaccine was effective in preventing WHV antigenemia in newborn when challenged with infectious virus. Disappointingly, only 13% of control pups developed chronic infection. Early experiments suggest that perinatal transmission of WHV is not the major route in this animal model. Experiments are underway to determine the route of transmission that leads to the development of the chronic carrier state. The usefulness of the woodchuck as a model for human HBV infection and sequelae depends on the induction of the carrier state at a high frequency. Routes of inoculation, age of pups at challenge, and dose of inoculum are currently being investigated.

The Pekin duck virus and ground squirrel virus are currently under study by NIAID grantees. Drs. Jesse Summers and William Mason have demonstrated that the duck hepatitis virus (DHV) replicates by reverse-transcription of an RNA intermediate. To this extent they are superficially similar to RNA tumor viruses. Whereas the tumor viruses use a tRNA to prime reverse transcription, the hepatitis B-like viruses appear to use a protein. For this reason, as well as the fact that the genomes of hepatitis B-like viruses are DNA rather than RNA, it is believed that they form a tumor virus family distinct from the RNA tumor viruses.

The development of a hepatitis A virus (HAV) vaccine appears to be a reachable goal. Tissue culture propagation of the virus has permitted experiments on growth and replication previously not possible. The intramural program has developed an attenuated clone of HAV for possible use as a live vaccine. The use of recombinant DNA techniques has resulted in the isolation of approximately 99% of the HAV genome as cDNA. Use of these clones should give rise to specific antigens and reagents useful for vaccine evaluation and diagnostic products.

The Hepatitis Program continues to support facilities and activities relative to chimpanzee breeding and experimentation for hepatitis viruses. These efforts in collaboration with the intramural program, provide an underpinning for characterizing the viruses and their interactions with a primate host. Studies are currently underway with HBV, HAV, non-A non-B viruses and delta agents. The chimpanzee is the only animal shown to be susceptible to all of the human hepatitis viruses and will be a resource much in demand for the foreseeable future.

Antiviral Substances Program

The Antiviral Substances Program is concerned with the development and preclinical and clinical testing of chemoprophylactic/therapeutic agents for control of viral infections. While vaccines remain the most effective way for preventing viral infections, this approach is not always feasible. Despite much recent activity in the area of development of a vaccine for herpes simplex virus infections, it is not yet clear that this approach will be effective in controlling the disease. Even when an effective vaccine becomes available, as with hepatitis B, effective antiviral agents are still required for those populations already suffering from chronic infection.

A major problem encountered in the development of safe and effective antivirals is the host cell toxicity that is frequently associated with their use. The discovery of a compound such as acyclovir, which depends on a viral induced enzyme for its activation, offers considerable hope that the therapeutic index of such agents can be increased. This is already obvious when acyclovir is compared to earlier agents such as iododeoxyuridine. Several newer drugs, which also depend on the HSV induced thymidine kinase for activation, are being evaluated in the animal models supported by the program. These are bromovinyldeoxyuridine (BVDU), 2'-fluoro-5-idoarabinosyl-cytosine (FIAC) and its metabolites 2'-fluoro-5-idoarabinosyl-uracil (FIAU) and 2'-fluoro-5-methyl-arabinosyl-uracil (FMAU), and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG), an agent that so far appears unique in that it has demonstrated antiviral activity in vivo against cytomegalovirus (CMV). Although these agents appear very promising in vitro and in vivo systems, only later pharmacokinetic and toxicologic studies will be able to evaluate their potential usefulness in man.

Multicenter collaborative clinical trials are an essential requirement for the proper evaluation of new agents in diseases such as herpes encephalitis, neonatal herpes and varicella zoster. These types of studies have been supported for several years with the University of Alabama at Birmingham serving as the Central Unit. Ongoing studies in both encephalitis and neonatal herpes are designed to compare adenine arabinoside (ara-A) to acyclovir (ACV). Recently the FDA has approved ara-A for herpes zoster in the immunosuppressed individual. The next protocol to be implemented will be a comparison of ara-A and ACV for treatment of disseminated zoster infections in the immunosuppressed. Although both of these drugs have been evaluated independently with early treatment of localized disease, no data are available on treatment of disseminated disease. Visceral complications develop in 60-80% of patients with disseminated zoster, and early therapy appears essential for these individuals. This study will test whether treatment can be delayed and still be effective. Two other studies are being planned by this group. One is designed to evaluate ambulatory therapy of zoster in immune suppressed populations (intramuscular ara-AMP vs. oral ACV); the second will attempt to treat the immune competent host where post-herpetic neuralgia is a common, and often severe, sequella of infection. The Central Unit is also continuing its attempt to devise a diagnostic test of herpes encephalitis infection which could replace the current mandatory brain biopsy.

Interest in the potential usefulness of interferon as a treatment for genital herpes virus infections has grown with the wide publicity that the current "epidemic" has received. The program initiated a clinical trial of human leukocyte interferon for initial infection in females in 1980. Due to the large number of cases of initial infection caused by HSV type 1 (which has a low recurrence rate) this study has been extended to obtain sufficient patients to evaluate the effect of therapy on both the initial infection and the incidence of recurrences.

Interferon is also being evaluated for treatment of laryngeal papilloma in children, an infection caused by a papilloma virus. Anecdotal reports from other studies continue to sound promising, and this large, controlled study should provide a definitive evaluation of interferon's effectiveness. This disease has been associated in some cases with genital condyloma in the mother. Anecdotal reports of the positive therapeutic effect of interferon in this condition are increasing. The disease is extremely common; physician consultations in 1981 were 946,000 compared to 295,000 for genital herpes. Condyloma infection in

females has been associated with cervical dysplasia and cancer. The program proposes to award a contract to evaluate several types of interferon for treatment of condyloma infection late in FY83.

Although most of the grant-supported work in this program concerns studies on interferon, several investigators are evaluating antiviral drugs. In studies that complement the work being performed in some of the animal model contracts, various new and licensed antivirals are being tested in combination in both *in vitro* and *in vivo* mouse models. Other workers have shown that esterification of ara-A makes the molecule more lipophilic, thus allowing it to pass membranes more easily and resist deamination. Thus, ara-ADA (the 2',3'-diacetate) is as active as acyclovir against herpes infections in both guinea pigs and mice.

Various investigators are involved in examining the structure and expression of both human and mouse interferon genes. Evidence continues to accumulate from Rockefeller Univ. (Sehgal) that multiple distinct human interferon beta genes exist.

An additional function of the program is to make available interferon reference reagents. This year saw the completion of an international collaborative titration of the newest interferon standards, which now include human gamma interferon and a cloned leukocyte preparation.

Bacterial Vaccines Program

Pneumococcal Diseases: Since the licensure of pneumococcal vaccine in 1977 and the completion of trials of pneumococcal vaccine for the prevention of otitis media, the NIAID pneumococcal vaccine program has gradually decreased in size. The present program consists primarily of collaborative studies of pneumococcal vaccine in various patient populations at high risk to pneumococcal infections. Although no NIAID funds are expended directly for these collaborative studies, they are made possible because of contract support of a reference laboratory for performance of pneumococcal antibody assays located at the State University of New York, Downstate Medical Center, Brooklyn. Several of the studies in this collaborative program have been completed and the results indicate that immunosuppression, either as the result of treatment or caused by the disease itself, is more important in determining response to the vaccine than the condition itself. For example, patients with Hodgkin's disease before treatment respond quite well to the vaccine. After immunosuppressive treatment, however, the antibody response is quite poor. Depending upon the individual condition and the degree of immunosuppression, the response varies within these extremes.

As mentioned above, the trials of pneumococcal vaccine in the prevention of otitis media have been completed during the past year and data analyses completed for most of them. The three trials in the United States sponsored by NIAID and the two trials in Finland sponsored by Merck Sharp & Dohme provide data which allow the following conclusions: (1) The ability of infants to respond to pneumococcal polysaccharides with the production of serum antibody is very poor. This ability to respond improved with age and the improvement varies with the individual polysaccharides. (2) Once an individual is capable of mounting a strong antibody response (attaining a level estimated at around 300 ng antibody N/ml) against a particular polysaccharide, protection against a pneumococcus of that type can occur. (3) Vaccination of children over two years of age offers approximately 50% protection against type specific disease but only about 10%

protection against overall otitis media. In this case, protection lasts only about six months to one year following vaccination. (4) Most children under two years of age, certainly those under one year of age, receive no protection from pneumococcal polysaccharide vaccines. (5) Effective and practical vaccination against pneumococcal otitis media will await development of vaccines more immunogenic in infants. Vaccines which are conjugates of protein and polysaccharide show great promise for prevention of Haemophilus influenzae type b meningitis in infants. If vaccines of this type are successful, pneumococcal conjugate vaccines will be developed for use in infants.

Meningitis: Approximately 20,000 cases of bacterial meningitis occur in the U. S. each year, mostly in young children and infants. For much of the past decade, program efforts have been directed toward development of vaccines for the protection of infants in whom most of this disease occurs. The major effort is in the area of Haemophilus influenzae type b disease. A purified capsular polysaccharide (polyribose phosphate, PRP) vaccine, developed with NIAID support, has been shown to be non-immunogenic and ineffective in infants. Various other approaches have been taken in developing a better infant immunogen including extraction of outer membrane proteins, combining the polysaccharide with DTP and the use of cross-reacting polysaccharides from other bacteria. The most promising approach, however, appears to be one in which the polysaccharide or oligosaccharide subunits are chemically conjugated with proteins such as diphtheria toxoid. One commercial manufacturer (Connaught) has developed a PRP-diphtheria toxoid conjugate which has stimulated excellent antibody response in one year old children; studies in infants down to 2 months old are underway. Another vaccine developed by an NIAID grantee and contractor (University of Rochester) is a conjugate of PRP subunits with either diphtheria toxoid or the analogous protein from CRM-197. CRM-197 is a diphtheria mutant elaborating a protein immunologically identical to diphtheria toxoid but which is non-toxic. The latter preparation has stimulated good antibody responses in one year old children and in two very young infants.

Lederle Laboratories has developed a vaccine of PRP mixed with DTP which has been immunogenic in infants and, through NIAID contract support, are isolating subcellular components of the pertussis organism to determine if a fraction responsible for the adjuvant activity can be obtained. An outer membrane protein has been extracted which appears to have the same adjuvant activity as the whole pertussis cell and clinical studies are underway on a vaccine made of this material and PRP.

During FY83, the NIAID will award a contract for the performance of an efficacy trial of a new H. influenzae type b vaccine for use in infants. The vaccine to be used in the trial will be selected from those mentioned above following completion of all safety and immunogenicity studies.

Research efforts on meningococcal vaccines are gradually phasing down. An NIAID sponsored efficacy trial has shown that group A polysaccharide is effective in preventing disease in infants. Group C meningococcal polysaccharide vaccine, although effective in adults and older children, is poorly immunogenic in infants. Efforts to improve this vaccine will probably follow the approach outlined above for H. influenzae vaccine if this proves successful. Likewise, this approach is planned (and underway in laboratories of commercial manufacturers) for group B meningococcal disease. The pure polysaccharide of the Group B meningococcus is non-immunogenic and efforts to produce a combined polysaccharide-outer membrane protein vaccine, although continuing, do not look promising.

Pertussis Vaccine: The current whole-cell pertussis vaccine, although effective in preventing disease, is sometimes responsible for undesirable side effects and research efforts are underway to develop an acellular vaccine. An acellular vaccine consisting of two major cell proteins has been developed in Japan and has been in use in that country for a number of years. The NIAID has taken two approaches toward the development of a new acellular vaccine for use in this country. The Institute has offered the use of its vaccine evaluation units for studies of the safety and antigenicity of any candidate preparations developed by commercial manufacturers. It has not been possible to obtain Japanese vaccine as Japanese manufacturers are concerned about liability. One American manufacturer has obtained bulk vaccine from a Japanese manufacturer and plans are being made to evaluate this material in NIAID evaluation units. In addition, other manufacturers are working on their own acellular vaccine, and informal discussions with them have begun regarding future testing.

In addition to this effort, the NIAID recently advertised for a contractor to develop an "American" version of this acellular vaccine. Proposals are currently being evaluated and an award will be made during FY83. It is anticipated that from two to four years will be required via either of these approaches before candidate pertussis vaccines will be ready for efficacy trials. Due to high vaccine acceptance and low disease rates in this country, efficacy trials could not be done here. Informal discussions have been carried out with investigators in the United Kingdom and in Sweden about future efficacy trials in those countries.

Viral Vaccine Program

The Viral Vaccine Program covers those areas where research should be stimulated toward the goal of an effective vaccine, but are not covered by the other specific programs. As previously reported, New York University is evaluating an attenuated varicella vaccine, under contract, in immunosuppressed children. Humoral and cellular immune responses in fifteen normal adults have been analyzed. To date 188 children and approximately 25 adults have been vaccinated; there have been 17 documented household exposures in leukemic vaccinees. Three have developed a mild chickenpox. The only possible side effect has been that a few children - with chemotherapy suspended - developed maculopapular lesions about one to two weeks after vaccine. Varicella virus was not isolated from them, nor has virus shedding been documented in any vaccinee; however, there is some indication of spread to susceptible siblings who had silent seroconversions. All vaccinees tested have responded immunologically.

The Development and Applications Branch also supports four General Vaccine Evaluation Units that form the backbone of the Branch's efforts for approaches to the control of important disease problems. Each unit has specialized population groups available to them, i.e., normal adults, pediatric groups, "at-risk" adults and/or children, for evaluating viral vaccines, bacterial vaccines, antiviral drugs, and environmental controls. Epidemiologic studies performed by several of the units have provided much of our current knowledge on the natural history of several of the respiratory agents. During the past year the evaluation units have participated in the testing of candidate vaccines in adults and children and also tested antivirals such as amantadine, rimantadine and interferon.

ANNUAL REPORT 1983

MOLECULAR MICROBIOLOGY AND PARASITOLOGY BRANCH

The Molecular Microbiology and Parasitology Branch plans and conducts research grant, program project grant, contract, training grant, fellowship and career award programs in molecular microbiology (biochemistry, genetics, DNA recombinants, physiology) and parasitology and medical entomology. It also coordinates the activities of the Parasitic Diseases Panel of the U.S.-Japan Cooperative Medical Sciences Program.

Approximate Level of Support

<u>Activity</u>	<u>Number</u>	<u>Amount</u>
<u>Molecular Microbiology Section</u>		
Research Grants 1/	217	20,880,057
Research Contracts	1	100,000
Training Grants	3	288,505
Fellowships	21	359,384
	<u>Subtotal</u>	<u>242</u>
<u>1/ Includes 17 Career Awards (\$756,811)</u>		<u>21,627,946</u>

Parasitology Section

Research Grants 2/	213	20,468,605
Program Project Grants	2	794,131
Research Contracts	2	296,656
Training Grants	6	440,409
Fellowships	14	230,328
	<u>Subtotal</u>	<u>237</u>
<u>2/ Includes 4 Career Awards (\$139,487)</u>		<u>22,230,129</u>

Branch

Research Grants 3/	430	41,348,662
Program Project Grants	2	794,131
Research Contracts	3	396,656
Training Grants	9	728,914
Fellowships	35	589,712
	<u>TOTAL</u>	<u>479</u>
<u>3/ Includes 21 Career Awards (\$896,298)</u>		<u>43,858,075</u>

STAFF ACTIVITIES

Dr. Delappe was invited to speak on the subject of the evolution, convergence and support for two seemingly unrelated programs (recombinant DNA and Biological Regulation of Vectors) at the 3rd International Colloquium on Invertebrate Pathology held September 6-10, 1982, at the University of Sussex, Brighton, United Kingdom. This was published in the proceedings of the colloquium.

Dr. Delappe obtained the services of Dr. Paul Englund of The Johns Hopkins University as a keynote speaker at the annual meeting of the Mid-Atlantic

Extrachromosomal Elements meeting and then introduced him to the audience. The meeting was held at Virginia Beach, Virginia (November 12-14, 1982).

In December 1982, Dr. Delappe was invited to attend the 14th Annual Conference of the Society of Vector Ecologists at the University of California (Riverside Campus). Budget uncertainties and the subject of the meeting--insects of agricultural importance--prompted him to decline the invitation.

An invitation was received by Dr. Delappe to give a lecture at the 5th International Symposium on Plasmids and Antibiotic Resistance at the Castle Smolenince, Bratislava, Czechoslovakia (September 4-8, 1983). Subsequent to submission of the abstract, an invitation was extended to give an introductory lecture at this symposium.

In early FY 1983, Dr. Delappe's special lecture during the 3rd Tokyo Symposium on Microbial Drug Resistance (October 23-25, 1981) was published in book form: "Drug Resistance in Bacteria"--Japan Scientific Societies Press, Tokyo and Thieme-Stratton, Inc., New York.

On February 8, 1983, Dr. Delappe represented the branch as a resource person during the Small Business Innovative Research Conference held on the NIH campus.

In late February 1983, Dr. Delappe received an invitation from the U.S.S.R. Academy of Sciences to be their guest for a period of 12 days in the Soviet Union (July 7-20, 1983). During the 12-day period no expenses except travel to and from the U.S.S.R. will be incurred by the U.S. Government.

The above was invited to attend the 5th Annual Meeting of the Great Neglected Diseases of Mankind Network sponsored by the Rockefeller Foundation at Swope Center of the Marine Biology Laboratory at Woods Hole, Massachusetts (September 28-October 3, 1982). No expense was incurred by the U.S. Government in conjunction with attendance at this meeting.

Dr. Delappe took an after working hours NIH course on plasmids (September 29, 1982-January 31, 1983).

Dr. Sheffield attended the 31st Annual Meeting of the American Society of Tropical Medicine and Hygiene, November 7-11, 1982, in Cleveland, Ohio.

Dr. Sheffield represented the Institute as Parasitology Program Officer at the meeting of the Parasitic Diseases Panel, U.S.-Japan Cooperative Medical Science Program in Bethesda, Maryland, July 18-20, 1983.

MOLECULAR MICROBIOLOGY

Program Summary

In addition to the basic free-ranging research of relevance to the Institute, there are two structured programs supported by this section of the Branch, one of which involves mechanisms of resistance to antimicrobial agents and the other, recombinant DNA.

(1) Mechanisms of Resistance to Antimicrobial Agents

During the past thirteen years, the problem of microbial resistance to therapeutic agents has become increasingly apparent. The widespread and indiscriminate use of antibiotics with associated increases in resistance of gram-negative and other organisms make the management of hospital-associated infections increasingly difficult. Of growing concern is the increase in penicillinase-producing gonococci.

There is substantial clinical and epidemiological evidence that development of antibiotic resistance, and especially plasmid (extrachromosomal) mediated drug resistance, represents a growing problem in medical care. Most of the projects are concerned with defining the fundamental biological mechanisms involved in the development of drug resistance by microorganisms. Specific goals involve investigation of the origin, development, evolution, expression and mechanism of drug resistance in a variety of specific microorganisms. Examples of microorganisms of particular interest are (but not limited to): Haemophilus influenzae, Enterobacteriaceae, Pseudomonas, Neisseria, staphylococci, streptococci, mycobacteria, mycoplasmas, and pathogenic fungi.

Research Highlights

AI 16805-04 R. K. Holmes (Uniformed Services University of the Health Sciences): Dr. Holmes et al studied immunoprecipitation and partial characterization of diphtheria toxin-binding glycoproteins from the surface of guinea pig cells. Diphtheria toxin (DT), a protein of molecular weight 63,000, is produced by Corynebacterium diphtheriae organisms that are lysogenic for phage carrying the tox gene. This toxin is synthesized as a single polypeptide chain that can be proteolytically cleaved to yield two fragments, A and B, which remain associated via a disulfide bond.

Intoxication of susceptible cells by DT is a complex process that involves the binding of the toxin, through fragment B, to specific receptors on the cell surface, followed by the translocation of the enzymatically active fragment A into the cytoplasm. Fragment A then catalyzes the transfer of ADP-ribose from nicotinamide-adenine dinucleotide to elongation factor 2 (EF-2), resulting in an inactive EF-2 and the cessation of protein synthesis.

Dr. Holmes provided direct evidence for the binding of DT to specific glycoproteins from the plasma membranes of guinea pig lymph node cells that are susceptible to the action of DT. These glycoproteins are not detectable in the cell membranes of mouse L cells that are resistant to DT. In addition, the binding of DT to these cell membrane glycoproteins is dependent on the presence of a functional fragment B with receptor-blocking activity. All of these observations suggest that the specific DT-binding glycoproteins detected may be physiologically relevant plasma membrane receptors for DT.

AI 02353-25 B. W. Catlin (Medical College of Wisconsin): This worker completed a study of the nutritional requirements and responses to 13 antibacterial drugs of 70 strains of N. gonorrhoeae isolated from patients in the 1940s--before penicillin was available for treatment of gonorrhea. These gonococci were typically susceptible to 12 of the drugs, but displayed a wide range of responses to sulfadiazine. Many strains were relatively resistant, a reflection of the heavy usage of sulfonamide drugs. Unexpectedly, most sulfonamide-resistant gonococci

required methionine for growth in a chemically defined medium. Overall, 39 of the 70 strains required one or more of ten compounds, but uracil or other pyrimidines were not required. This finding suggests that the profile of requirements for arginine, hypoxanthine and uracil which is common in recent isolates from disseminated gonococcal infections probably evolved after treatment with sulfonamides was replaced by penicillin. The nutritional requirements and antibacterial resistance of a group of 97 gonococcal strains isolated from Milwaukee patients in 1975 were determined using methods and materials similar to those applied in the study of gonococci isolated in the 1940s. Consequently, the findings exemplify the evolution of gonococci. Only one strain required methionine, but 18 required arginine, hypoxanthine, and uracil. Some strains were resistant to penicillin and other drugs. On the other hand, some gonococci were inhibited by vancomycin, rifampin, and erythromycin in concentrations lower than those inhibitory for strains of the 1940s.

Hypersusceptibility is of practical importance because the culture media routinely used for the laboratory diagnosis of gonorrhea contain vancomycin in concentrations of 3 to 4 ug/ml, together with other compounds that normally inhibit commensal bacteria but not gonococci. The inability of vancomycin-hypersusceptible gonococci to grow on the diagnostic medium may lead to the failure to treat infected persons and the consequent risk of the development of complications as well as continued transmission of gonorrhea.

(2) Recombinant DNA Molecular Research

During the past few years, the development of certain techniques in the area of molecular biology has made it possible to construct functional DNA molecules in vitro which contain segments derived from diverse biological sources. The scientific innovations which led to this technological breakthrough were mostly derivative from basic studies on the mechanism of restriction, which normally acts as a barrier to gene flow among microorganisms, and on the molecular biology and genetics of bacterial plasmids, especially those specifying drug resistance. Grantees of this Institute played a preponderant role in both of these areas. Whereas DNA recombination in nature has depended on random processes, the experimental techniques now available enable the in vitro construction and subsequent replication of DNA molecules needed for specific experimental goals. This basic advance, coupled with refinements of the existing technology, should provide greater knowledge of the mechanisms of pathogenicity (at the molecular level) of viral, bacterial, mycotic and parasitic agents. This information, in turn, may lead to improved prevention, diagnosis and treatment of infectious diseases. Notwithstanding the potential benefits of recombinant DNA molecule research, there may be associated potential biohazards which must be avoided by the design, construction and testing of safer host-vector systems for use in these studies.

The primary goal of this research is the development and utilization of recombinant DNA molecule technology to increase fundamental knowledge and ultimately enhance control of the etiological agents of infectious diseases. Another goal is the synthesis of a variety of biologically useful substances through the construction of bacterial cells containing functional DNA of either plant or animal origin. An equally important goal is the identification, assessment, and elimination of any and all potential biohazards encountered in the exploitation of this technology.

Research Highlights

AI (F32) 06480-02 S. Lory (Harvard Medical School): The mechanisms by which Gram-negative bacteria, with double membranes, export proteins to the exterior are not known. While it has generally been assumed that the proteins pass through both membranes, an alternative possibility is that they move laterally from the cytoplasmic surface of the inner membrane, through a connecting junction, to the external surface of the outer membrane, from which they might be released. Dr. Lory has been studying this problem in Pseudomonas aeruginosa, since it secretes a wider variety of proteins to the exterior than E. coli, and he has worked extensively on the biochemical aspects of one such secreted protein, exotoxin A.

He has found that exotoxin A of this organism does not normally accumulate significantly in the cell, but in the presence of membrane perturbants, such as ethanol, a larger precursor accumulates, and it is all found in the outer membrane. This precursor has been identified through both its immunological and enzymatic activity. He has also studied the secreted enzyme phospholipase C of P. aeruginosa and has cloned its structural gene in E. coli. The new host synthesizes but does not export the enzyme, and most of it remains associated with the outer membrane; for lack of antibodies he has not yet been able to determine whether it accumulates as a larger precursor. Since two normally secreted proteins accumulate in the outer membrane under conditions that block secretion, the model of lateral transfer followed by cleavage from the outer membrane seems attractive. This work is being continued.

Further work with the cloned plasmid has indicated that phospholipase C is an 80,000 dalton polypeptide, since insertion mutagenesis at a unique restriction site eliminated the production both of this polypeptide and of phospholipase C activity in cell extracts. This work was the beginning of his use of recombinant DNA to study protein secretion. Further understanding of the organization and the expression of the genes now requires more refined techniques, including DNA hybridization and DNA sequencing with site specific mutagenesis.

AI 08619-15 S. Cohen (Stanford Medical School): During the current year, he has continued to make progress on his studies of gene expression in Streptomyces. Promoter-probe plasmid vectors were constructed for Streptomyces lividans, using expression of the E. coli chloramphenicol acetyltransferase gene as an indicator of promoter activity. These vectors were used to isolate and to study the activity of DNA sequences that contain transcriptional control signals from Streptomyces, Bacillus licheniformis, E. coli, and Serratia marcescens. Studies of these promoter regions in heterospecific hosts indicate that genus or species-specific factors may present barriers to the expression of bacterial genetic material in certain heterologous cellular environments. While promoter regions isolated from E. coli, S. marcescens and B. licheniformis all appear to be recognized by the RNA polymerase of S. lividans, the Streptomyces transcriptional control signals isolated do not appear to function normally in E. coli.

(3) Other Basic Research

AI 01278-26 and AI 04769-21 K. Rinehart (University of Illinois): On the first of his two grants funded by NIAID, the less active but most abundant antiviral/antitumor agent (didemnin A) from the Caribbean tunicate Trididemnum sp. has been successfully converted to the most active but less abundant didemnin B.

Preparatory to a total synthesis of didemnin A, the sub-unit N,O-dimethyltyrosine has been prepared in quantity, as have some potentially useful di- and tripeptide intermediates.

On the second grant, during the past year he has continued his studies of the antiviral, antitumor didemnins. Didemnin B, which is scheduled for clinical trials as an antitumor agent, has been shown to be active *in vitro* and/or *in vivo* against a number of lethal RNA viruses, including Rift Valley fever virus and a Pichinde virus related to Lassa fever virus, and is also strongly immunosuppressive. A number of new didemnins have been isolated, including didemnins D and E, potential precursors to didemnin B.

Among other biologically active marine natural products, structures have been assigned to the major antifungal components of two related Caribbean sponges, a new class of antibacterial compounds, consisting of tetritol p-hydroxybenzoates, has been identified in a California mollusk, and the structure of hexahydro-polyandrocarpidine has been revised to a substituted pyrrolidone, which has been synthesized. He has also isolated and characterized a series of intensely antiviral compounds from a Caribbean colonial tunicate.

R22 AI 12522-28 C. Ballou (University of California, Berkeley): Dr. Ballou continues his studies on complex polysaccharides in Mycobacterium sp. The general objectives of his research project are to define the synthetic pathways and functions of the polymethylpolysaccharides (PMPS) that he discovered and that are found only in mycobacteria. These polysaccharides, composed either of 3-O-methylmannose or 6-O-methylglucose, interact in a specific manner with long-chain fatty acids and acyl-coenzyme A derivatives, and they act as lipid carriers in this microorganism. They probably allow the organism to make the large lipids that are incorporated into the cell wall and protect the bacteria from lysosomal attack. He expects that it may be possible to alter the resistance of mycobacteria to lysosomal attack if one could interfere with the synthesis of their lipids. This might be done by perturbing the polymethylpolysaccharide lipid carrier function, and these studies should lead to feasible ways of doing this. In his search for precursors of the methylglucose polysaccharide, he has discovered a new class of glycolipids in Mycobacterium smegmatis. These compounds have 4-5 hexose units and they contain pyruvic acid as well as long-chain acyl groups. One goal will be to complete the characterization of these compounds, to determine where they are localized in the cell, and to investigate their possible role as antigens. He plans to characterize apparent precursors of methylglucose polysaccharide that become labeled when M. smegmatis cells are pulsed with [³H] methylmethionine or [¹⁴C] glucose. These substances are acidic, as they should be if they contain glyceric acid, and they contain glucose and 6-O-methylglucose, but they are somewhat smaller than mature methylglucose polysaccharide molecules. Thus, he thinks they might be incompletely methylated precursors, and this is supported by the fact that they are degraded to smaller fragments by amylase digestion.

PARASITOLOGY

Program Summary

In addition to the basic free-ranging research of relevance to the Institute, there are two structured programs supported by this section of the Branch, (1) biological regulation of vectors and (2) immunology of parasitic infections.

(1) Biological Regulation of Vectors

This program has as its goal the advancement of fundamental studies which might lead to effective methods of biological regulation of vectors. For the past 30 years, control of pests and disease vectors has been based primarily on the use of synthetic organic compounds which had the "advantages" of long residual action and toxicity to a broad spectrum of target organisms. It has now been shown that, because of these very characteristics, many of these pesticides are more deleterious than beneficial when all effects on man and his environment are considered. Furthermore, resistance to broad spectrum chemical pesticides has reduced their effectiveness in many vector control programs. For these reasons the search for alternative methods of pest control has become imperative, and it is generally agreed that the best approaches will consist of integrated pest management programs which combine biological control, in the broadest sense, with the judicious use of more specific chemicals and management of the physical environment. This approach to vector control must be based on adequate information about the ecology of the target organisms in which the control program is to be conducted, effects of control measures on non-target organisms in the environment, and the biology of the disease organism being transmitted.

Research Highlights

AI 17736-02 T. Bradley (University of California): In a study of cellular defense mechanisms of mosquitoes, Dirofilaria immitis infection of Malpighian tubules in mosquitoes has been examined. In Aedes sp. the apical microvilli of Malpighian tubule cells atrophy and the mitochondria retract from them whereas in Anopheles quadrimaculatus, where mortality is high, the microvilli do not change but the interior of the cell becomes necrotic. This suggests that reduced transport by the tubule membranes is not the cause of death. These results were further supported by measurement of fluid secretion of Malpighian tubules in vitro which showed reduced secretion in susceptible and resistant strains of Aedes but not in Anopheles. Further comparison of susceptible and resistant mosquito strains is in progress.

AI 12772-04 B. Federici (University of California): Coelomycetes dodgei, a fungus parasitic on mosquitoes, undergoes gametogenesis resulting in the formation of gametes which mate and form zygotes that seek out and kill mosquito larvae. The most critical factor controlling gamete release is the onset of the dark period during the final 24 hours of gametophyte development. Neither the onset nor length of the last light period, nor the length of the last dark period significantly affects the initiation of gametogenesis or gamete release in comparison to the effect of the last dark period. By manipulating the last dark period, up to 80% of the gametes can be induced to be released within one hour. These results are significant in that they will permit highly synchronous production of mosquito-infective zygotes once the fungus is cultured in vitro.

AI 15886-04 A. Spielman (Harvard University): Human babesiosis is transmitted by the tick, Ixodes dammini. Babesia microti penetrates the tick gut epithelial cells initially by invagination of the host membrane which then disintegrates. Two structures, an arrowhead and the coiled structure are implicated. Upon contact with the epithelial cell the arrowhead undergoes changes and finally disappears after penetration which supports the suggested role of enzyme release by this organelle. The coiled structures undergo changes ending in myelination

and possible enzyme release. These studies relate B. microti to typical Babesia organisms and to erythrocyte-dwelling organisms such as Plasmodium sp.

(2) Immunology of Parasitic Infections

The complexity of structure and function of parasites has made the study of the immunology of these infectious agents exceptionally challenging and rewarding. Exciting opportunities for the elucidation of mechanism and manifestations of immunological responses to parasites now exist as the result of the impressive developments in immunology in recent years. Major ultimate goals of studies on the immunology of parasitic infections are the development of effective vaccines for the prevention of parasitic diseases (such as malaria, schistosomiasis, and filariasis), the intervention in the host response to prevent or ameliorate disease processes which are immunologically mediated, and the development or improvement of immunodiagnostic procedures for parasitic infections, especially as they relate to the immune status of the host.

A related goal of these studies is to contribute to an understanding and solution of basic and clinical problems associated with other disease entities, especially immunological disorders and hypersensitivity states. A number of parasitic infections are excellent models for such studies as (a) the mechanisms of intracellular immunity, (b) the enhancement of suppression of concurrent infections or tumor development, (c) immunopathological mechanisms, (d) development of disease process in immunosuppressed or immunostimulated hosts, (e) the biochemical and genetic mechanisms for the development of pathogen variants with different immunological characteristics, (f) the genetic basis for variations in host response, and (g) the role of IgE and other cytotropic antibodies in hypersensitivity.

Research Highlights

AI 17309-03 N. Agabian (University of Washington): This investigator is studying the molecular basis of antigenic variation in African trypanosomes. Using refined methods for the immunological detection of cloned gene sequences from either cDNA or gDNA libraries using monoclonal, polyclonal or patient antisera she has identified variant antigen expressing cDNA clones. Characterization of these reveals a novel codon usage in the translation of variant antigen mRNA and further indicates that even proteins made from mRNA which contains significantly different codon preferences from E. coli are nevertheless expressed in detectable amounts.

AI 11289-10 D. Colley (Vanderbilt University): Studies on the interrelationships between the host's immune response system and Schistosoma mansoni during chronic murine schistosomiasis have shown immunoregulation of granuloma formation to be mediated by a soluble T lymphocyte-derived suppressor factor. This activity is titratable and can be administered to mice to modulate their granulomas. The factor is anti-idiotypic and expresses an I-J determinant of the Major Histocompatibility Complex. Its characteristics indicate that it is part of the T suppressor cascade seen in well-defined hapten-specific T suppressor systems. Regulation in the schistosome egg granuloma appears to be quite similar to a variety of well-studied immune systems.

AI 02631-25 F. von Lichtenberg (Harvard University): Although feasibility of immunization with a live, attenuated vaccine for schistosomiasis is being evaluated in several laboratories relatively little is known about the local and systemic host reactions. When both radiation-attenuated and non-attenuated

schistosomula were given to mice by intramuscular injection, an relatively intense and prolonged reaction occurred. Parasites were usually destroyed within 7 days. Fibroblast proliferation and muscle regeneration began by day 3 and, by 4 weeks, inflammatory infiltrates and regenerative proliferation underwent gradual involution with injections sites being healed with no scarring by the end of 4-5 weeks. These studies show that attenuated schistosomal vaccines cause marked local inflammatory responses but no systemic lesions and their injection sites heal without permanent damage.

AI 18873-02 J. Manning (University of California): Major and minor proteins present on the surface of Trypanosoma cruzi epimastigotes, metacyclic trypomastigotes, amastigotes, staphylomastigotes, bloodstream trypomastigotes and trypomastigotes derived from infected tissue culture cell lines have been identified. The results indicate that the surface protein profiles of epimastigotes and metacyclic trypomastigotes are indistinguishable and are distinct from that of blood stream trypomastigotes. Profiles for bloodstream and tissue culture derived trypomastigotes are identical. And, the major surface antigen recognized by antisera from hyperimmune mice is a 90 Kd protein which is present on the surface of staphylomastigotes, bloodstream trypomastigotes and tissue culture derived trypomastigotes. This protein is refractory to labelling on amastigotes suggesting that it is either masked by other cellular (possibly host) proteins or is internalized.

AI 17429-03 R. Nussenzweig (New York University): In the identification of a protective surface antigen of Plasmodium falciparum sporozoites, monoclonal antibodies raised against sporozoites reacted with polypeptides (circumsporozoite proteins) that are uniformly distributed over the entire surface. The epitopes recognized by the monoclonal antibodies were expressed on sporozoites from different geographical isolates of the homologous species but were not detected on sporozoites of heterologous species nor on blood forms of the parasite. Two polypeptides from extracts of sporozoites were immunoprecipitated by the monoclonal antibodies and by serum from successfully vaccinated human volunteers. These circumsporozoite proteins play a role in immune protection. Incubation of the appropriate monoclonal antibody with viable sporozoites of the homologous species significantly reduced parasite infectivity as determined by sporozoite neutralization assays carried out in splenectomized chimpanzees.

AI 18695-03 R. Reese (Scripps Clinic and Research Foundation): To learn more about the immunologically important components of the malarial parasite, this investigator isolated m-RNA from the ring, trophozoite, and schizont stages. Translation of this material with either the wheat germ or rabbit-reticulocyte lysate systems has allowed him to produce many of the same immunologically important peptides made by the organism during its normal in vitro cultivation. Furthermore, the peptides made under these conditions were immunologically active, thus the epitopes were contained in their peptide structures without processing or glycosylation. The obvious importance of this is that once these structures are determined, peptide chemistry can be used to inexpensively synthesize these antigens.

AI 17615-02 D. Wyler (Tufts University): Egg granulomas of Schistosoma mansoni, macrophages from granulomas, and schistosomal eggs produce biologically active molecules which can stimulate fibroblasts in vitro. This activity resides in molecules of 15,000 daltons. It also has been shown that bovine vascular smooth muscle cells and endothelial cells also proliferate in vitro to similar molecules.

These findings provide strong circumstantial evidence that hepatic fibrosis in schistosomiasis - characterized by periportal fibrosis, vascular smooth muscle hyperplasia, and endothelial proliferation - is due to biologically active molecules elaborated by cells in the granulomas. These studies also provide a model which may explain the pathogenesis of tissue fibrosis in other diseases associated with chronic inflammation, including lymphatic filariasis.

AI 15503-04 T. Yoshino (University of Oklahoma): The objective of this project is to determine the molecular basis for resistance and susceptibility of genetically defined stocks of the snail, Biomphalaria glabrata, to Schistosoma mansoni. In a comparison of hemocyte surface antigens and lectin-binding sites utilizing monoclonal antibodies, it was shown that snail hemocyte populations represent a molecularly heterogeneous group of cells which exhibit both intra- and interstrain differences. Investigations on antigen sharing between schistosomes and snails have revealed a high degree of antigenic similarity between schistosome larvae (miracidia, primary sporocysts) and the snail (serum, hemocytes). Such antigen sharing patterns gives some indication that coevolution of this parasite-host system has involved selection for molecular similarity in some major snail and schistosome surface components.

(3) General Parasitology Research Highlights

AI 17340 C. Bacchi (Pace University): Polyamine synthesis and metabolism has been shown to be an important factor in metabolism and reproduction of African trypanosomes. These parasites are highly vulnerable to polyamine inhibitors. Activity of many standard trypanocides as well as novel agents are influenced by co-administration of polyamines. D,L-alpha-difluoromethylornithine (DFMO), a direct inhibitor of polyamine biosynthesis, is successful in blocking division of trypanosomes and can cure infections in vivo. DFMO acts synergistically with bleomycin, an antitumor agent, to deplete polyamines. Such activity with antitumor agents as well as known trypanocides may be effective in treatment of the various forms of African sleeping sickness.

(4) Contract Activity

The schistosomiasis supply contract at the University of Lowell has continued to provide all three human schistosomes (Schistosoma mansoni, S. japonicum and S. hematobium) and their vector snails (Biomphalaria sp., Bulinus sp. and Onchoceraria sp.) to 49 schistosomiasis researchers on 306 occasions during the last contract year, August 3, 1981, to August 2, 1982. Sufficient inventories of parasites and snails are continuously maintained.

The filariasis supply contract at the University of Georgia has filled 162 requests for research materials during the last contract year, February 22, 1982, to February 21, 1983. Five species of parasites (Brugia pahangi, B. malayi, Dirofilaria immitis, Dipetalonema viteae and Litomosomoides carinii) and the vectors Aedes aegypti mosquito eggs and Ornithodoros tartakovskii ticks were shipped. Breeding colonies of rats and dogs produce animals to serve as parasite hosts. Sufficient inventories of parasites and vectors are continuously maintained. Materials supplied by this contract have greatly facilitated the acceleration of research on filariasis.

EXTRAMURAL ACTIVITIES PROGRAM

NIAID

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ANNUAL REPORT
October 1, 1982 through September 30, 1983
Director's Report
Extramural Activities Program
National Institute of Allergy and Infectious Diseases

The Extramural Activities Program (EAP), National Institute of Allergy and Infectious Diseases (NIAID), serves as the Institute focal point for the administration and fiscal management of the research grant, contract and manpower programs. The structure of EAP includes components responsible for scientific merit review, grants management, contracts management, committee management, manpower development and research grant support services including processing of applications.

The EAP is responsible for the initial review for scientific and technical merit of contract proposals, and applications for program projects, centers, clinical investigator awards, and conference grants, and the second level review for Individual National Research Service Awards. The EAP also carries out the overall coordinating and supervisory function with respect to the planning and preparation for the National Advisory Allergy and Infectious Diseases Council (NAAIDC), and the Institute's Research Contracts Advisory Group (RCAG) and Fellowship Review Group (RFG). It also has the responsibility for the processing and issuance of contract and grant awards in the respective scientific program areas. Further the EAP studies, performs and/or oversees other extramural activities, e.g. preparation of summary data, analysis of study section behavior, analysis of manpower programs. A separate function performed by the EAP is the distribution of reference reagents together with information and technical advice on their characteristics and use.

The Director, EAP, in consultation with the Office of the Director, NIAID, works closely with Program Directors and other Institute staff regarding questions of policy relating to the Institutes extramural programs.

The senior staff of EAP is made up of:

Director
Deputy Director
Chief, Program and Project Review Branch
Chief, Grants Management Branch
Chief, Contracts Management Branch
Manpower Development Officer
Head, Research Resources Section
Special Assistant to the Director

The following personnel actions were processed in FY 1983:

Appointments

Dr. John W. Diggs, Director,
Dr. William E. Bennett, Chief, Research Manpower Development Staff
Dr. James Ferguson, Executive Secretary, Program and Project Review
Branch
Ms. Patricia Havenstein, Contract Specialist, Contract Management
Branch
Nancy M. Hershey, Contract Specialist, Contract Management Branch
Dorothy C. Hinden, Fellowship Technical Assistant, Research
Manpower Development
Nadine Tyler, Clerk Typist, Committee Management Office
Eilene J. Unsworth, Procurement Assistant, Contract Management
Branch

Departures

Mary H. Mace, Contract Management Branch
Celeste Mayer, Contract Management Branch
Carole K. Shapiro, Committee Management Office
Sara M. Spencer, Contract Management Branch

Awards

Mary Jane Lucas, Program and Project Review Branch

In summary, The Extramural Activities Program provides administrative, scientific, fiscal, and support services to the Institute Director, Program Directors as well as to other extramural staff.

A. Committee Management

The Committee Management Office continued to function in an exemplary fashion. This office maintains overall responsibility for management and coordination of activities related to Institute committees, boards and advisory groups.

Five members completed their terms on the National Advisory Allergy and Infectious Diseases Council as of October 31. Six new members were on board for the January 1983 meeting - one replacing an earlier resigner. The nomination slate for three new members with terms beginning November 1 has been submitted.

The term of office for one member was extended for two years and a second person was invited to serve a four year term on the Board of Scientific Counselors as of July 1, 1983.

The name of the Microbiology and Infectious Diseases Advisory Committee was changed to Microbiology and Infectious Diseases Research Committee. One member's term was extended for a year and four new members were invited to join the Committee as of July 1.

The Allergy, Immunology, and Transplantation Research Committee reduced its membership from 19 to 18 members. Seven new members have been nominated to join the Committee as of July 1.

The charters for all four Committees were renewed for one year as of July 1. Normally charters are granted for a two year period, but NIH is trying to adjust the number of charters renewed each year. This means that the NIAID Committees were rechartered for one year and must go through the process again in 1984.

B. Conference Proposals

The number of R13 conference grant applications assigned to NIAID by the Division of Research Grants in FY 1983 remained essentially the same as in FY 1982. NIAID received primary assignment on twenty-seven R13 applications. Initial scientific review was conducted by our Institute's three chartered committees. These applications, along with fifteen conference grant applications having NIAID as a secondary assignee, were subsequently reviewed en bloc by Council. Following Council review, recommendations for the NIAID funding contributions were made by the Research Contract Advisory Group (RCAG) to the Director for his final approval (TABLE I). Support for conference grant proposals approved for funding increased from \$80,000 in FY 1982 to 89,228 in FY 1983, while the number of awards (eighteen) remained the same. Five awards totaling \$46,500 were made by the Immunology, Allergic and Immunologic Diseases Program (IAIDP); thirteen awards totaling \$42,728 were made by the Microbiology and Infectious Disease Program (MIDP).

Sixteen of the conference grants funded in FY 1983 received support through co-funding assistance awards. That is, the Division of Research Grants (DRG) arranged for joint sponsorship of conferences by two or more (average = 3) interested BIDs through multi - Institute assignment and review. To make certain conference grant applications were brought to the attention of all interested BIDs, the Director, Extramural Activities Program (EAP) actively assisted Program staff in obtaining additional assignments and in soliciting dollar support when appropriate. Every effort has been made by EAP staff to develop and maintain viable procedures for the processing of scientific meetings funded totally, or in part, by the NIAID through the R13 conference grant mechanism.

TABLE I

NUMBER OF R13 CONFERENCE GRANT APPLICATIONS REVIEWED BY NIAID,
FY 1983*

Action	AI Primary	AI Secondary	TOTAL
Approved for Funding	12	6	18
Approved at Fundable Priority, not approved for Funding	2	4	6
Approved at Unfundable Priority or Disapproved	13	5	18
TOTAL	27	15	42

*September 1982, January 1983 and May 1983 Councils

C. Transfer of Research Grant Applications to other BIDs

By negotiating transfers of assignment to the Institute most capable of making an award, Institute staff best serve the interests of the scientific community. The number of pending applications transferred by NIAID to other BIDs has doubled during fiscal year 1983. For the most part, this increase has been a reflection of NIAID's funding capability, and not of the relevance of the proposed research to the Institute's programmatic interests. In addition, the development and maintenance of a paper tracking system by the Referral Liaison has contributed to the effectiveness of monitoring these transferral actions.

Table 2 shows the number, by Program, of pending applications that have been transferred to other BIDs for funding consideration during the past fiscal year. The National Cancer Institute continues to request the largest number (39% in FY 1983) of these transferrals.

TABLE 2

GRANT APPLICATIONS TRANSFERRED BY NIAID
TO OTHER BIDS
FOR FUNDING CONSIDERATION, FY 1983*

Awarding BID	IAIDP	MIDP	Number Total
CA	18	-	18
GM	6	7	13
AM	4	3	7
HL	3	-	3
HD	2	-	2
AG	1	-	1
DE	-	1	1
RR	-	1	1
TOTAL	34	12	46

* September 1982, January 1983, and May 1983 Councils

D. Study of Priority Scores and Study Section Behavior

In response to a request by NAAID Council, the Special Assistant to the Director, EAP, has monitored data relating to the scoring characteristics of the Division of Research Grants Study Sections that review approximately 84% of the research project applications assigned to the Institute (ALY, BM-1, BM-2, EVR, IMB, IMS MBC-1, MBC-2, TMP, VR). By observing the distribution of priority scores (175 or better) since the January 1981 Council round, significant differences have been noted in the rating behavior of these ten review groups. Statistical tabular presentation of data shows, what appears to be, random fluctuations of behavior, both vertically (Study Section to Study Section) and horizontally (Council to Council). The number of variables influencing scoring behavior are not known, but it can be assumed that these variables probably act in combination(s) as well as independently. The "easy" (+) and "hard" (-) raters were identified by determining the deviation from an NIAID mean approval rate for research project grant applications receiving a priority score less than, or equal to, 175. During the last three Council rounds, deviations from the NIAID mean ranged from +21 (MBC-2) to -23 (BM-2).

The percentile system is a procedure for minimizing, or "normalizing", the inequities in scoring behavior exhibited among review groups. In determining the percentile ranking of an approved application, the priority scores of the current and two preceding meetings of the Study Section is used as the base against which a priority score is compared. This is done for all of the approved applications, regardless of Institute assignment. A comparative analysis of the data has demonstrated the effect of priority score versus percentile ranking on making funding decisions. This study has shown that approximately 90% of approved research applications with priority scores above the payline would be funded by either method. Therefore, only 10% of the fundable applications, or on the average twelve per round, fall into a zone near the payline where funding is dependent on the system used. Because of the variation in Study Section behavior percentile ranking would allow funding of applications with priority scores up to 30 points beyond the present payline. This process, as presented, does not recognize areas of programmatic interest or scientific potential and opportunity.

E. Research Manpower Development

During the fiscal year, one hundred and sixty-seven (167) competing individual fellowship applications (F32's) were reviewed; support is budgeted for 54 fellows which results in an award rate of 32 percent.

TABLE 3

Applications Reviewed and Awarded by Degree

Total Reviewed <u>Applications</u>	Number Reviewed		Number Awarded		Total Awarded <u>Applications</u>
	<u>Applications by Degree</u>	<u>M.D.</u>	<u>by Degree</u>	<u>M.D.</u>	
167	39 (4)*	128 (3)*	9 (1)*	45 (1)*	54
100%	23%	77%	5%	27%	32%

* Senior Fellows (F33) in parenthesis

Table 3 reveals that physicians received 17 percent (9 of 54) of all awards and, as a group, had an award rate of 23 percent (9 of 39). Holders of the academic doctorate received 83 percent (45 of 54) of all awards, an award rate of 35 percent (45 of 128). Approximately one-fourth of the awards were to females who realized an award rate of 24 percent. Fellows will be trained in more than 40 different institutions located in 9 of the 10 DHHS regions.

Table 4 is a summary of individual fellowship support for new competing and continuation grants by extramural program area.

TABLE 4

Individual Fellowship Awards

Program Area	Continuation and Supplemental Awards				Total		
	New Awards	Number	Amount*	Number	Amount*	Number	Amount*
IAIDP				21	\$420,000	42	\$803,000
MIDP				36	660,000	28	528,000
TOTAL	57	\$1,043,000		49	\$948,000	106	\$1,991,000

* Includes both direct and indirect costs

The total number of awards has varied only slightly (106 to 113) during the last three years as a result of budgetary constraints. This has substantially limited the degree to which the fellowship program can be made to be responsive to the new national concerns surrounding Acquired Immunodeficiency Syndrome and Sexually Transmitted Diseases for there are continuing high priority unmet manpower needs in other fields as reflected in Table 5.

TABLE 5
FY 1983
Number of Applications Awarded
by Discipline

Discipline	Degree				TOTAL
	M.D.		Ph.D.		
	Male	Female	Male	Female	
ANATOMY					
Cell Biology			1		1
BIOLOGY					
Molecular Biology			5	(1)*	6
CHEMISTRY					
Biochemistry			2	1	3
Organic Chemistry			2		2
Inorganic Chemistry			1		1
GENETICS					
Genetics			5		5
MICROBIOLOGY/IMMUNOLOGY					
Microbiology			4	2	6
Immunology			5	6	11
Parasitology			1	1	2
Virology			7	1	8
INTERNAL MEDICINE					
Infectious Diseases		3			3
Immunology					
TOTAL	5	(1)*	1		6
	8	1	33	12	54

* Senior Fellows (F33) in parenthesis

There is an obvious need to train additional manpower in fields where long-standing shortages have existed: especially allergy, clinical immunology, tropical medicine and infectious disease epidemiology in addition to those mentioned earlier.

Also recognized is the need to develop the many potential biomedical scientists in the relatively untapped pool of under-represented minorities. The Institute targeted support for research and manpower development in this area through its cooperative efforts with the National Institute of General Medical Sciences (NIGMS) and the Division of Research Resources (DRR). One continuation and two competing applications were funded under the Minority Access to Research Careers

Program, NIGMS and two competing projects were funded under the Minority Biomedical Research Support Program, DRR.

Undergraduate professional students represent the unique pool from which future clinical investigators will be developed. One competing grant was awarded to a school of veterinary medicine and one continuation grant to a school of allopathic medicine under the Short-Term Health Professions Training Program; combined these grants will support the training of 49 health professional students in the coming academic year.

I. Contract Management Branch

The Contract Management Branch (CMB) provides management services to the Institute's Research Program including solicitation, negotiation, award, and administration of all Institute research contracts.

In the past year there has been turnover in contract specialists. New staff includes Ms. Nancy Hershey and Ms. Patricia Havenstein who replaced Mrs. Sara Spencer and Mrs. Mary Mace.

The CMB continues to implement contract policies and procedures promulgated by higher procurement authority. The CMB works very closely with the Program and Project Review Branch and the various NIAID Project Officers and provides contract management expertise necessary in order to continue to have an effective contract program.

Through effective contract management, NIAID has continued to increase its percentage of competitive awards from 26.2% in FY 1978 and 37.3% in FY 1979, to 60% in FY 1980, to 67% in FY 1981, 76% in FY 1982 and an estimate of 80% in FY 1983. NIAID is one of the few Institutes which exceeded the competitive goals established by Division of Contracts and Grants.

Other accomplishments for FY 1983 are:

1. Led all Institutes in competitive and scheduling goals for FY 1981, 1982 and 1983 which are established by PHS.
2. Even distribution of contract awards within the fiscal year.
3. Made contract awards in all three socioeconomic programs; 8(a), Small Business Administration and women-owned organizations.
4. Continues to lead all Institutes (in total percentage) in meeting the goals established by Division of Contracts and Grants in closing out of contracts.

In FY 1983, CMB received approval for 12 contract requests and issued about 689 requests for proposals to organizations which expressed interest. In response to the above advertisements, 90 proposals were received and 21 new competitive contract awards were made.

II. Grants Management Branch (GMB)

• In Fiscal Year 1983 the GMB issued more than 2,000 grant and cooperative agreement awards for approximately \$196 million. This represented approximately 72% of the Institutes total budget of \$273.6 million. By way of comparison, 1982 NIH grant and cooperative agreement awards totaled \$2.541 billion or 69.7% of the total NIH budget of \$3.643 billion. The GMB is responsible for the fiscal and administrative management of all grants and awards issued by the NIAID. The GMB works very closely with program staff and provides the fiscal and administrative expertise necessary for effective program management. The GMB also serves as an interpreter of grant policy and procedure issued by the several echelons within the DHHS.

• In FY 1983 the NIH operated under a Continuing Appropriations. Regular research grant awards prepared early in the fiscal year, with 8% reductions, had to be revised upward to reflect budget reductions of 5%. Generally, for all research awards, the award level was reduced by five percent from the committed/approved level. Actions on other types of awards were as follows:

- Individual Fellowships (F32's) were prepared reflecting \$3,000 for the Institutional Allowance (normally \$5,000).
- Institutional Training Grants (T32's) were prepared reflecting allowances of \$2,500 per postdoctoral trainee and \$1,500 per predoctoral trainee (normally \$5,000 and \$3,000 respectively).
- Career awards (K4's, K6's, K7's and K8's) were not reduced.

Award notices prepared in FY 1983 did not reflect any reductions in future period commitments. However, it remains to be seen as to whether or not we will be able to honor the full commitments in FY 1984 and beyond. Further, with regard to FY 1984, it is anticipated that indirect cost awards, for research grants, may be made at less than the full amount. There are a number of options under consideration as to the method to be used in effecting the reduction(s).

• To provide an update on issues cited in previous reports, shown below are the issues with comments as appropriate:

Non-categorical Financial Status Report (FSR)

The FSR has now virtually fully replaced the categorical Report of Expenditures (ROE), and while the FSR clearly does not provide detail to our liking, we have adapted by requesting, from the grantee, detailed information on a case-by-case basis.

Project-period segment concept

The ability to use "savings" from one project-period segment to help fund another continues to aid the institute in meeting existing commitments as well as in the funding of competing initiatives.

• NIH Directors Advisory Committee

- Extensions (liberalization) of Institutional Prior Approval System

The NIH convened work group (of which the Chief, GMB was a member) recommended, in fact, that current delegation of authority under the IPAS be expanded to include certain prior approval authority that currently is vested at the NIH. The PHS is presently considering the work group recommendations and, as a result, may conduct a study of their own.

- Automatic Carryover of Unexpended Direct Costs

The NIH convened work group recommended that automatic carryover not be adopted. To allow automatic carryover would severely reduce the "savings" that accrue to NIH annually. Further, the NIH currently has the capability of approving carryover on a case-by-case basis.

- Transfer of Costs Between/Among Related Projects

The recommendation of this NIH convened work group is still under consideration by EFMC, but the work group has essentially recommended that the NIH continue to allow - and even promote to a higher degree of incidents - transfers between and among related projects as long as they meet the test of relatedness.

- Limited Trial of the Fixed Obligation Grant (FOG)

The NIH convened work group recommended that FOG not be adopted, not even on a trial basis. They felt that a trial was not practical. They also concluded that FOG carried with it some features of automatic carryover that would further erode our potential savings.

Again in FY 1983, staff of GMB were able to make many valuable contributions, both as members and chairpersons, of policy and procedure work groups, committees and subcommittees at the NIAID and the NIH. The Chief, GMB was Director of a new STEP Module entitled "R&D Procurement: Perceptions, Practices, and Pitfalls". Additionally, the Chief, GMB participated as a faculty member at the "NIH Grants Administration Conference" held in Houston, Texas (October 1982) and Burlington, Vermont (June 1983), and attended by representatives from more than three hundred grantee institutions.

During FY 1983, the QMB which in FY 1982 was formally reorganized from a single branch with no formal sub-structure to a single branch with three formal sections, operated smoothly and efficiently with no staff turnover. The Branch, with the respective Section Chiefs and their staff is comprised thusly:

SECTION

SECTION CHIEF

Grants Management Officer (Chief, GMB) Mr. Gary Thompson
Ms. Joyce Lopez (Secretary)

<u>SECTION</u>	<u>SECTION CHIEF</u>
Immunology Grants Management Section	Mrs. Mattie Tynan* Mr. Joe Kirby Mr. William Mitchell
Microbiology Grants Management Section	Mr. Todd Ball Ms. Karen Steinberger Ms. Lois Eggers Ms. Barbara Huffman
Grants Support Section	Ms. Marietta Robinson Mr. Rick Wiener Mr. Hubert Sumner Ms. Cindy McDermott Mrs. Libby Hall Mrs. Gertrude Cohen Ms. Patricia Coates Mrs. Martha Decker Mrs. Shirley Caplan

*Also serves as Deputy Chief, GMB.

It is expected that the reorganization of GMB will continue to further the objectives and better facilitate the significant interactions between and among Grants Management, Program, and Review.

• There were two new grant initiatives in FY 1983 that are certainly worth mentioning.

• The NIAID became involved with Cooperative Agreements for the first time. In the Spring of 1983 we issued four cooperative agreements in response to the RFA entitled "Studies of Acquired Immuno-Deficiency Syndrome (AIDS) Kaposi's Sarcoma and Opportunistic Infections".

• In response to P.L. 97-219, which was an amendment to the Small Business Act requiring PHS (and certain other federal agencies) to set aside a specified amount of their R&D budgets for a Small Business Innovative Research (SBIR) Program; in late FY'83, the NIAID awarded approximately fourteen grant awards (PHASE I) to what was, essentially, a totally new grantee constituency.

• The GMB also, in April 1983, had four representatives (Mr. Ball, Mrs. Huffman, Ms. Steinberger, and Mr. Mitchell) visit the University of North Carolina and Duke University for an intensive three-day learning experience geared toward learning more about our grantee constituency. Conversely, in July 1983, we hosted a three-day visit by two representatives from the Office of Grants and Contracts at Duke University. This "exchange" program was very helpful in further improving the "partnership" that exists between the NIAID and our grantees.

"In FY 1983, as in the past, employees were encouraged to broaden their horizons beyond their day-to-day routine and fill spots created by temporary absences. Classes, seminars, and STEP programs were attended by most staff members. In general the staff of GMB continued to move forward in their individual development while maintaining a high level of excellence in pursuit of the day-to-day duties and responsibilities that encompass all grant programs funded by the NIAID.

III. Program and Project Review Branch

A. Review Committees

During the current year, both the Allergy, Immunology and Transplantation Research Committee (AITRC) and the Microbiology and Infectious Diseases Research Committee (MIDRC) had heavy workloads reviewing contract proposals, and applications for program projects, centers (AIRC), clinical investigator awards, and conference grants. While there was no difference in the workload of the AITRC between 1982 and this year, there was a remarkable increase in the MIDRC review load. The MICRC increase was primarily due to readvertisement of the RFA concerning the International Collaboration on Infectious Disease Research (ICIDR) Program. The response to the latter consisted of 20 program project grant applications and 22 "Part B" (Developmental), or R21, applications necessitating the convening of two ad hoc committee meetings in addition to the regularly scheduled MIDRC meeting at the February/March 1983 cycle (the MIDRC reviewed the STD program projects at that time).

In recognition of the continuing heavy review workload borne by only two executive secretaries in the Branch, Dr. Diggs requested from the Director, NIAID, another professional slot (with an accompanying slot for support staff), for a third executive secretary for the Branch. The request was granted, and the Executive Secretary for Special Review, came on board July 1. Dr. James Ferguson will be responsible for ad hoc reviews of proposals not assigned to the chartered committees, the review of conference grants; along with the other executive secretaries, he also will be conducting site visits. The addition of another executive secretary is particularly timely in view of increased initiatives in the contract arena. NIAID is promoting activities on accelerated development of vaccines. In addition the current public health emergency, AIDS, is resulting in increased activities involving scientific review.

To remind NIAID staff of the NIH policy concerning separation of review from management and to maintain objective peer review, the Director, NIAID, has issued a policy statement clarifying program staff/review staff/committee roles.

Also being implemented at review meetings is a method suggested by the Director, NIAID, which permits reviewers the opportunity to rescore applications if it seems apparent from the Chairman's point of view that sufficient consensus had not been reached.

B. Review Services Unit

The Unit, which is responsible for processing all grant applications and corresponding summary statements assigned to NIAID, remains understaffed in relation to the continuously increasing workload handled by the Unit (see table below). During peaks of activity, i.e., 2-3 weeks before Council meetings, it has become routine for RSU staff to work daily on overtime basis. Often the Review Services Unit has to recruit other support staff outside the Unit to assist in the preparations for Council. The lack of space is an obvious and ever growing problem, and a space management analysis has been conducted under the auspices of the Office of the Executive Officer to study the situation.

Number of Grant Applications
Processed in the Review Services Unit
1977 through 1983

Council	1977	1978	1979	1980	1981	1982	1983
January	580	605	549	553	652	729	787
May	601	687	734	751	840	948	1,002
October	653	616	611	794	739	645	

C. Microbiology and Infectious Diseases Research Committee

The Microbiology and Infectious Diseases Research Committee (MIDRC) held three meetings: October 1982, February 1983 and June 1983. In addition, two Ad Hoc meetings were held to review grant applications in reply to RFA 82-1, "International Collaboration in Infectious Diseases," to which some committee members had submitted applications. The unusually high number of responses to this RFA also necessitated the additional review meetings.

Grants: The MID reviewed six program project applications in response to an RFA for "Sexually Transmitted Diseases Centers." Of these applications, four were approved and two were disapproved. The committee reviewed 19 institutional training grants, one clinical investigator award application, 17 conference grant applications and one program project supplement. All of the training grant applications were approved; the program project supplement was disapproved. Only 11 of the 16 conference grant applications were approved and the clinical investigator application was disapproved.

An Ad Hoc review group reviewed 20 program project applications in response to RFA 82-1. Of these 13 were approved and 7 were disapproved. A second Ad Hoc group reviewed the 22 proposals submitted in response to Part B of the RFA. Of these 20 were approved and 2 were disapproved.

The total grant dollars reviewed (01 year) were \$18,710,049 and the total approved were \$8,658,258.

Contracts: The full MIDRC reviewed six contract proposals involving two RFP's and one noncompetitive renewal proposal. Five proposals were approved; two proposals and the noncompetitive renewal were awarded. The total dollars competitively reviewed by MIDRC for contracts was \$2,363,106 and the total amount awarded was approximately \$656,000.

Five Ad Hoc meetings were conducted to review 35* contract proposals in response to five RFP's. One proposal was awarded for each of the RFP's, except for RFP 83-11 for which two or three proposals may be funded.

A program presentation was made at the October MIDRC meeting describing the ICIDR program.

Concept clearance was granted for RFP-83-6 "Maintenance of a Repository and Distribution Center for Reagents."

Table 6 shows the workload of the Committee in 1983 compared with 1982.

*Estimating that 20 proposals will be received for RFP 83-11.

TABLE 6
WORKLOAD OF THE MIDRC, FY 1982 AND FY 1983

	<u>NUMBER OF APPLICATIONS/PROPOSALS REVIEWED</u>	
<u>CONTRACTS</u>	<u>MIDRC 1982</u>	<u>MIDRC 1983</u>
Solicited (RFP Responses and Renewals):		
Full Committee	28	7
Ad Hoc	9	35*
Unsolicited	0	0
<u>Total Contract Proposals</u>	37	42*
<u>GRANTS</u>		
P01/P50		
Solicited (RFA Responses)		
Full Committee	6	6
Ad Hoc	0	20
Unsolicited	1	1
Supplements	1	1
R21 (RFA Response) Ad Hoc	0	22
T32	21	19
K08	3	1
R13	16	16
<u>PROJECT SITE VISITS (P01/P50)</u>	7	1
<u>PROGRAM EVALUATION</u>	0	0
<u>CONCEPT CLEARANCE</u>	0	1
<u>TOTAL GRANT PROPOSALS</u>	48	86

* estimated. The response to RFP-83-11 will not be known until July 11.
The figure assumes 20 responses.

D. Allergy, Immunology and Transplantation Research Committee, NIAID

The Allergy, Immunology and Transplantation Research Committee (AITRC) is composed of two subcommittees, the Allergy and Clinical Immunology Subcommittee (AIRC), and the Transplantation Biology and Immunology Subcommittee (TIC). The AIRC reviews programs and projects in allergy, clinical immunology and immunopathology, while the TIC is primarily concerned with programs and projects in immunobiology, immunogenetics, immunochemistry and transplantation biology. These committees provide initial review of grant applications and contract proposals for special programs which include program projects and centers, institutional training grants, conference grants and special developmental programs in the areas mentioned above. In addition, the subcommittees assess the progress, evaluate ongoing special programs of the NIAID and advises on program developmental activities in these areas.

Allergy and Clinical Immunology Subcommittee

Application/Proposal Review: The AIRC met three times during fiscal year 1983 and reviewed 39 grant applications which included 21 program projects and centers, 13 institutional training grants, 3 clinical investigator awards and 2 conference grants (Table IV). Thirty-five of these applications were approved. The total dollars requested in these applications and approved by the subcommittee were \$53,672,116 and \$20,181,678, respectively. The program project and center applications reviewed were in the areas of Mechanisms of Immunologic Diseases, Asthma and Allergic Diseases, and in Interdisciplinary Research in Immunologic Diseases. In accordance with the recommendation of the AITRC, none of these applications were site-visited.

Program Advice: The program staff sought the AIRC's advice on a variety of issues including hybridoma cell lines and data bank, food allergy, drug allergy, immunotherapy for asthma and various other immunologic diseases.

Concept Clearance: The Subcommittee cleared a concept entitled, "Clinical Trials: Efficacy of Immunotherapy for Asthma."

E. Transplantation Biology and Immunology Subcommittee

Application/Proposal Review: During its three meetings in FY 1983, the TIC reviewed 11 grant applications which included 1 program project in lymphocyte biology, 6 institutional training grants, 1 clinical investigator award and 3 conference grants (Table 7). All of these applications were approved and total dollars reviewed and approved were \$5,202,203 and \$2,669,108, respectively. In addition, 34 Master Agreement proposals were reviewed by an ad hoc review group (Table IV). Twenty-three of these proposals were found to be acceptable. These Master Agreement proposals were received in response to the RFP-NIH-NIAID-IAIDP-83-2, entitled, "Develop and Supply Serologic Reagents for the Investigation of Polymorphic Human Cell Surface

Antigens (Master Agreement)". The current estimate is that another group of 5 contract proposals will be submitted in response to the RFP-NIH-NIAID-OSD-83-9, "Histocompatibility Typing for Molecular Studies of Disease Susceptibility Genes." These proposals will be reviewed by an ad hoc review group of the TIC in August, 1983.

Program Advice: Program staff sought advice from the TIC on a variety of issues including hybridoma bank, serum bank, bone marrow transplantation and kidney transplantation.

Concept Clearance: The Subcommittee reviewed and approved four program concepts. These relate to serum bank and clinical trials of appropriate monoclonal antibodies in kidney and bone marrow transplantation.

TABLE 7

REVIEW WORKLOAD OF THE AIRC AND TIC, FY 1982 AND FY 1983

TYPES OF APPLICATIONS	AIRC REVIEW LOAD		TIC REVIEW LOAD		AIRC & TIC TOTAL REVIEW LOAD	
	FY82	FY83	FY82	FY83	FY82	FY83
P01/P50	19	21*	15	1	34	22
T32	15	13	0	6	15	19
K07	2	0	0	0	2	0
K08	10	3	0	1	10	4
R13	9	2	8	3	17	5
N01	0	0	17	39**	17	39
Program Concept Clearance	<u>2</u>	<u>1</u>	<u>0</u>	<u>4</u>	<u>2</u>	<u>5</u>
SUBTOTAL	57	40	40	54	97	94
Site Visits	<u>9</u>	<u>0</u>	<u>11</u>	<u>0</u>	<u>20</u>	<u>0</u>
GRAND TOTAL	66	40	51	54	117	94

*One P50 application was reviewed by a Special Review Group.

**Thirty-four Master Agreement proposals were reviewed by an Ad Hoc Review Group. It is estimated that 5 contract proposals will be received in response to RFP-NIH-NIAID-OSD-83-9 in FY 1983.

IV. Research Resources Section

A. Introduction

The need for well-characterized reference reagents as an adjunct to research is well recognized. In support of this concept, the Research Resources Section (RRS) conducts a program which distributes the reagents with information and technical advice on their characteristics and use. The RRS is responsible for the management of operations including distribution and cataloging of the microbial, allergen and some of the immunologic reagents. Mrs. Sylvia Cunningham serves as the Head of this Section.

B. Allergen & Immunologic Reagents

The processing and packaging of five lots of hypersensitivity pneumonitis antigens and antisera were completed during the year under a procurement contract with the American Type Culture Collection. The final products were sent to the Mayo Clinic for final testing; all reagents were found to be satisfactory with the exception of the antiserum to *S. Viridis* which is too dilute at the recommended reconstitution volume. However, the certification laboratory has recommended that the product is still a worthwhile reference reagent and can be used with a modification of the reconstitution instructions. A note to this effect will be recorded on the appropriate catalog page and new labels will be ordered for this antiserum.

Final packaging and testing has been completed for the new lots of ragweed antigen extracts E and K. Catalog pages have been prepared and are currently being reviewed by the allergen reference center. A number of requests for the antigen E have been received; distribution of this item will be initiated as soon as the catalog pages have been finalized. Extraction of antigens Ra-3 and Ra-5 has now been completed by David Klapper, University of North Carolina. As soon as the recommendations for pooling and methods of diluting have been made, the Research Resources Section will proceed with the arrangements to have the two lots labeled and packaged.

Acquisition of the ten (10) food allergens was completed by the allergen reference center. The RRS assisted with obtaining proper forms and formats for the contractor to obtain the IND for the products. The food allergens are now being distributed to seven investigators currently participating in the study.

This Section has continued to closely coordinate the activities of the allergen reference laboratory located at the Mayo Clinic with the functions of this office. Coordination activities include scheduling of various tasks such as packaging, labeling and working out time schedules for distribution.

In addition to the activities listed above the RRS has continued to distribute other allergenic products such as rye grass I, II and III, venoms of honey bee, yellow-hornets, white-faced venom; these venom products are available for distribution under an IND as well as for in vitro use.

A new catalog describing the allergenic and immunologic reagents is currently being prepared. It is expected that this catalog will be available in August.

C. Microbiological Reagents

The intensive work of prior years has resulted in the completion of work on the enterovirus, adenovirus, rhinovirus, myxovirus and the agents and antigens of hepatitis A and B. In most cases, seed viruses preparations and corresponding antiserum are now available for most of the viruses of public health interest. The reagents which have resulted from the various projects have been most useful; particularly the hemagglutinins, neuraminidase, ribonucleoproteins and the influenza viruses of man and animals.

The Enterovirus Pools A thru H originally prepared in 1971 have been widely distributed during the past 13 years. Last year the supply reached a low level and alternate methods for preparation of a new lot were pursued. The method determined to be most feasible and least expensive was for the WHO to distribute the workload between two Enterovirus Collaborating Centers (Baylor University, Houston, Texas and the Serum Institute, Copenhagen, Denmark). Dr. Melnick at Baylor is currently reevaluating the bulk pools provided by NIAID. When the reevaluation has been completed, the bulk material will be combined into the final pools and sent to Copenhagen for packaging and storage.

The demand for interferon standards and antiserum continues to be high (interferon shipments currently represent 37% of the total reagent shipments). Several new items have been placed in the repository and will be released for distribution soon. The new items include human gamma standard and goat serum human beta interferon.

Work is now underway for the preparation of a special catalog listing only interferon standards. If enough data becomes available and the catalog can be completed by October, an information table will be set up at the Interscience Conference on Antimicrobial Agents & Chemotherapy meeting to be held October 23-26, 1983, in Las Vegas, Nevada.

D. Processing and Distribution

During this year a blanket type procurement agreement has been in effect for the processing and packaging of various items. In addition to the materials mentioned above, this contract has assisted with some of the packaging requirements of the International Union of Immunological Society (I.U.I.S.). To date two small pilot lots of extracts have been processed for evaluation. It is expected that five additional lots will be completed by September 1983.

The RRS reagent collection consists of over 350 individual reagents. A tabular record of distribution since FY 1974 is presented in the attached table. The decrease in distribution can be attributed to the following two factors (1) the impact of the reagent transfer of the four largest viral groups to ATCC is now being reflected in the numbers of reagents available for distribution and (2) the change in distribution policy consisting of invoicing investigators a reagent handling fee and shipping charges. This procedure was implemented in an attempt to recover some of the costs for the reagent storage and distribution.

The reagent repository and distribution contract remains at Flow Laboratories; however, as the contract requirement was scheduled for recompetition this year and as a result of the reduction in some of the work requirements (primarily the HLA serum bank portion), the project has been designated for an 8(A) set aside. This program is a special contract program by which contracts are placed thru the Small Business Administration to small "socially and economically" disadvantaged firms. A facility has been located that has the required space and experience to perform the contract. A detailed proposal from the organization has been received, reviewed and approved by an adhoc review committee. During the remainder of this fiscal year and the during the first quarter of next year, plans will be developed to move the repository from McLean, Virginia to the new facility in Rockville, Maryland.

RESEARCH RESOURCES SECTION
DISTRIBUTION OF VIRAL, MYCOPLASMAL,
AND ALLERGEN REAGENTS

<u>Fiscal Year</u>	<u>Total Transactions</u>	<u>Total ampoules & vials distributed</u>
1974	500	9,932
1975	592	6,751
1976	762	10,188
1976 (TQ)	192	3,126
1977	613	7,633
1978	605	6,851
1979	782	16,223
1980	793	11,238
1981	912	11,096
1982	805	8,296
1983	450	4,900*

* Projected total for 1983. Actual figure as of 6/15/83 is 3,300

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Summary of Program
Laboratory and Clinical Research, NIAID
October 1, 1982 - October 1, 1983
Office of the Scientific Director

The Annual Report of the Intramural Research Program contains individual summaries of the research projects in 13 laboratories which constitute the research components of the National Institute of Allergy and Infectious Diseases. Administrative responsibility for the Intramural Research Programs resides with the Office of the Scientific Director (OSD). Ten of the laboratories are located at the NIH Campus in Bethesda. The three remaining laboratories are located in Hamilton, Montana, in a facility called the Rocky Mountain Laboratories.

We were fortunate to recruit Dr. James C. Hill to serve in the OSD as the Associate Scientific Director. He, along with Mr. Charles Criswell, the Administrative Officer, and Mr. Robert Steiner, Chief of the Rocky Mountain Operations Branch, provides senior administrative and management control over the Intramural Programs of NIAID. An additional off campus research facility in Building 550 at the Frederick Cancer Research Center has continued to expand its programs and has undergone major renovation to accomodate a new animal facility and expanded laboratory research facilities. This off campus operation is administered by Mr. Stan Nagle.

Expansion of intramural expertise in molecular biology continues to be an important program objective. Molecular biology programs have been added to the Laboratory of Immunogenetics with the recruitment of Dr. Eric Long and Dr. Edward Max. Dr. Anthony Fauci, Chief of the Laboratory of Immunoregulation has also been successful in recruiting a molecular biologist from Dr. Philip Leder's laboratory at Harvard. Dr. Ulrich K. Siebenlist will shortly join the staff to explore molecular biological problems in human immunology.

The Acquired Immune Deficiency Syndrome (AIDS) has provided a major challenge both scientifically and administratively to the Intramural Programs of NIAID. This disease is characterized by immunodeficiency particularly of T helper cells accompanied by a vast array of opportunistic infections which ultimately lead to the death of the affected patient. Because the syndrome involves both the immune system and multiple infections, it clearly fell under the scope of research being performed by NIAID and the Clinical Center. The majority of patients with AIDS who are admitted to the NIH Clinical Center are cared for in the NIAID program. Currently, almost 60 patients with AIDS are being followed. Attempts are being made to restore immune function through use of agents such as interleukin 2 and immune interferon. At the same time, Dr. Fauci and the Laboratory of Immunoregulation are studying the immune defects seen in these patients with the intent of identifying a correctable aberration in the immune system.

Many investigators are also examining ways to identify a new infectious etiological agent which may be responsible for AIDS. Dr. Robert Purcell of the Laboratory of Infectious Diseases has injected blood and cells from AIDS patients into chimpanzees in an attempt to transmit the infection responsible for AIDS. Dr. Hoggan and the members of the Laboratory of Viral Diseases have examined serum from patients to see if antibodies to parvoviruses may be

present in AIDS patients. A new laboratory was established in OSD with its primary responsibility the study of AIDS. Dr. Thomas Folks was recruited to supervise this program which has rapidly developed the capability to analyze immune complexes, immunosuppressive factors and immunologic reactivity in AIDS patients. The laboratory, however, is focused primarily on a study of chimpanzees who receive injections of AIDS tissues or blood to determine if changes develop which might indicate a transfer of infection. In addition, Dr. Folks, along with Dr. Chused of the the Laboratory of Microbial Immunity, has undertaken "two color" sorting on the fluorescent activated cell sorter to look at phenotypes of T cells which can be correlated with activation markers. Activation of T suppressor cells and NK cells have been detected using this approach. Almost 8% of the entire intramural budget and program resources have been redirected to the study of AIDS. This has been assisted by an additional allocation of 1 million dollars to the intramural program specifically designated for the support of AIDS research. A contract for nearly \$600,000 dollars was established with the New York Blood Center and Memorial Sloan-Kettering Cancer Center in New York to obtain specimens from individuals who are at risk of developing AIDS. Over 325 individuals will be followed. It is anticipated that several thousand specimens of blood, semen, urine, feces and saliva will be available shortly for those laboratories that are now developing programs for isolation of various viral, fungal, bacterial or protozal agents which may be responsible for the irreversible and fatal immunosuppression seen in AIDS patients. A sub-contract was awarded to the University of Texas Primate Center at Bastrop, Texas, to maintain chimpanzees that will be available for injection of AIDS tissue specimens. Another contract at Meloy Laboratories makes available six chimpanzees; some have already been injected with AIDS tissue material. In addition, the Rocky Mountain Laboratories in Hamilton, Montana, have begun a renovation of one of their buildings to provide future space for more than 20 chimpanzees which may be used for AIDS research or other intramural infectious disease research. Dr. Richard Wyatt serves as project officer on contracts established to provide AIDS specimens. He also has accepted the role of Executive Secretary of the Scientific Working Group on AIDS which coordinates the research of the intramural program of NIAID.

Four intramural research projects have been established within the Office of the Scientific Director to conduct research on AIDS. AI00383 is a project under the direction of Dr. Richard Wyatt which utilizes the contract at the New York Blood Center and the Memorial Sloan-Kettering Cancer Center to acquire specimens from individuals at risk of developing AIDS. A second, AI00384, describes the immunologic evaluation of AIDS carried out by Dr. Thomas Folks' laboratory in which specimens from AIDS patients are being used to affect the growth characteristics of normal T cell lines and clones. Functional changes in B cell activation following cryopreservation has been examined and a method for cryopreservation of B cells which is suitable for maintenance of both T cell and B cell function for future study of the immune defect in patients is being developed. Chemical and functional characterization of immune complexes is currently underway. Complexes have already been isolated and made available for DNA dot blot techniques with cDNA probes from potential AIDS infectious agents. Project number AI00385 describes the work of Dr. Folks and his laboratory, in studies of chimpanzees who have been exposed to tissues, blood and cells from AIDS patients. The whole array of immunologic testing being employed currently in the study of the AIDS patients has been applied to studies of chimpanzees. In the process

of performing these studies, Dr. Folks identified a T suppressor population in chimps that is not marked with the T subset marker. Functionally, these cells were shown to be natural killer cells. Similar populations have been identified in AIDS patients and may represent an activation or transition of T suppressor cells to natural killer cell activity. Finally, Dr. Robert Buller and Dr. Richard Wyatt are in the process of establishing a facility in which one can use conventional viral isolation techniques to look for parvoviruses and other viruses which may be present in AIDS patients and may be responsible directly or indirectly for the T helper dysfunction seen in this syndrome. This project, number AI00378, will not be fully implemented until the next fiscal year.

We continue to use the P4 containment facility in Building 41T on the NIH campus. Dr. Robert Buller and Dr. Gordon Wallace are nearing completion of studies of viral epidemiologic and biologic aspects of mouse pox (ectromelia) infections. They have been able to develop a sensitive assay for infection and to show the value of various preventive immunization techniques. They have also shown clearly the influence of genetic background on susceptibility to infection and the more difficult problem of transmission and persistent infection.

During the past year, Dr. Gordon Wallace who previously served as Assistant Scientific Director and as Head of the ectromelia research program in the Laboratory of Viral Diseases, was selected as the Director of the Life Sciences Program of the President's Committee on Science and Technology. This committee under the overall leadership of Dr. Keyworth is responsible for recommendations to the White House regarding national programs in both medical and physical sciences.

The "round robin" of renovation is nearing completion and the majority of scientists located in Building 8 have been moved to Building 5. All animals in Building 8 have been relocated either to Building 5 or Building 14B. Building 5 renovations are nearly completed although the malaria section will not be able to occupy their spaces until the spring of 1984.

The central services facilities of the intramural program of NIAID have operated to provide for a coordinated and cooperative program of research support. Peptide synthesis under the direction of Dr. Lee Maloy has been implemented to provide expert interaction with several scientists including those in the Laboratory of Molecular Microbiology, the Laboratory of Immunogenetics and the Laboratory of Infectious Diseases. Dr. Salzman, utilizing a DNA oligonucleotide synthesizer, has collaborated with individuals to prepare gene sequences for direct study. During the year, funds were available for the procurement of two new fluorescent activated cell sorters. One of these was installed this year; the second will be installed in the late fall of 1983. Dr. Myron Waxdal has agreed to join the Office of the Scientific Director to serve as the central scientific supervisor and liaison for the operation of fluorescent activated cell activities in support of research in all laboratories of the Intramural Program.

The Rocky Mountain Laboratories received an intense review by members of the President's Private Sector Survey on Cost Control. It had been suggested that this laboratory could be abolished or abandoned and the scientists either supported through extramural grants or transferred to the Bethesda Campus of

NIH. It was gratifying to learn through the process of review that the recent surge of research competence and capability at RML was recognized nationally and internationally by peer groups of scientists and scientific organizations. Several hundred letters were forwarded to the President's Private Sector Survey committee supporting the current work and direction of research activity of the Rocky Mountain Laboratory. This support along with a recognition of the NIAID's plans to continue to develop an area of research excellence at the Rocky Mountain Laboratories led the Private Sector Survey group to make a recommendation that the laboratory continue to be supported if the efforts for further development at the laboratory would be documented and fully supported by NIAID. The suggestion matches well with our activities over the past few years to provide new resources and personnel to RML and to expand the scientific base and critical mass of scientists located at this facility. The new electron microscopy laboratory is now fully operational and providing a major new capability to the RML scientists. The high quality of the work at RML is evident when the publication record of the scientists are examined. New technologies of recombinant DNA research as well as hybridoma monoclonal antibody development are now freely available to scientists at RML. Redevelopment of the Epidemiology Branch continues as wide recruitment for a new laboratory chief to serve in this area is now in progress. It is anticipated that this new laboratory will have its fundamental basis in molecular biology technology and will provide essential interaction with the virology, immunology and bacteriology programs already located at RML. The development of the laboratories, recruitment and support of scientific programs has been served in an outstanding way by Mr. Robert Steiner, Chief of the Rocky Mountain Operations Branch.

Commitment to recruitment of minority scientists in biomedical research continues to be evidenced by IRP support of the Introduction to Biomedical Research Program. For the fifth year, NIAID invited 50 science students to spend time at NIH being introduced to biomedical research as a possible career. These students were identified as outstanding candidates by the several hundred schools who were contacted. Many were selected to participate in the summer program which this year accommodated more than 90 students. Over 70% of the summer students were either minority or female. Dr. Katherine Cook Jaouni provides outstanding support for this program and for all of the participating students. While there is a continued shortage of Hispanic and native American employees at all levels, we are particularly aware of a shortage of all minorities in scientific positions in NIAID. NIH and NIAID have, however, made better progress than most universities in identifying and attracting women and minorities to careers in science. Nevertheless, our programs for minority recruitment continue to receive the highest level of priority in NIAID.

The animal care facilities of NIAID are finally settling into a stable position. Most of the animals in building 8 and 5 have been transferred to the newly renovated building 14B on the NIH campus. This building, as well as the Rocky Mountain facilities, meets fully the AALACL requirements for animal care. Our laboratories in building 5, 7 and the Clinical Center have been improved so as to also meet requirements of AALACL. We anticipate that all facilities of the animal care sections both at Bethesda and RML, as well as Building 550, Frederick, will be reexamined by AALACL recertification committees and that successful accreditation will be received.

This year the Scientific Director was active on several committees of NIH which impacted directly on intramural research operations. As a member of a committee to review central services support at NIH, the Scientific Director assumed direct responsibility for reviewing the Office of Scientific Research. This office provides all administrative and management support for central service activities including any operation of over 3,000 employees and also for approximately 1/3 of the management fund budget. The Scientific Director also participated in an evaluation of all upper mobility programs and took direct responsibility for an extensive study of the STRIDE Program at NIH. These studies have resulted in a recommendation which should make available upper mobility programs to the most deserving of NIH personnel in areas where they can best serve NIH interest. The Scientific Director also participated in a study of third party insurance payments for patients hospitalized in the clinical center. Through an extensive analysis of the insurance industry and its practices, as well as the clinical research programs at NIH a report was prepared which successfully defended the concept of non-reimbursable and non-insurance related hospitalization of patients for clinical research at NIH. The Scientific Director also serves on the Facilities Planning Committee which is responsible for the long-term master plan for development of the entire NIH facility and the development of new resources and new laboratories. As part of this activity, we have begun to outline the needs for a new microbiological research building on the NIH campus. The Scientific Director also served with a group organized by Surgeon General Koop for the study of organ procurement for transplantation in the United States. As part of this activity, OSD became responsible for planning a meeting for a study of the maintenance of donors to be used for multiple organ procurement.

NIAID continues to maintain a good safety record. More importantly, the intramural program and its scientists are committed to training and participation in safety development.

In order to provide better support for the scientists and the scientific management of the intramural programs, we plan to reorganize the OSD. With the start of FY 84, a management and administrative branch will be organized to collect all administrative, procurement, personnel, editorial, and management operations. Dr. James Hill will serve as the chief of this branch and will be assisted by the intramural administrative officer. Other portions of the OSD will be organized administratively to provide scientific support services to all members of the scientific staff. This new laboratory will contain sections dealing with the operation of the fluorescent activated cell sorter, peptide synthesis, DNA synthesis, animal care section, clinical immunology laboratory and support for AIDS research. This new laboratory should provide equal access for intramural scientists to new technologies which are important in the development of their research programs. It also will provide an economical way to provide high cost high technology within the constraints of the intramural budget.

The quality of the research operation received favorable recognition from the Board of Scientific Counselors who reviewed all of the intramural laboratories in the Clinical Center. The Laboratory of Immunoregulation, the Laboratory of Clinical Investigation and the Laboratory of Immunology were reviewed during this year. All were shown to be laboratories operating at the cutting edge of science and providing national leadership in the development of clinical investigation programs and the training of new young clinical investigators.

The laboratory of Immunology was commended as one of the outstanding cellular immunology laboratories in the world. Site visits by the peer review Board of Scientific Counselors resulted in a strong recommendation to continue the support of and even expand these programs as they provide new scientific information in important areas of biomedical research. At the same time, they are a national asset for the maintenance of a strong clinical biomedical research initiative.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00071-12 OD

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Pertussigen

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

J. J. Munoz Research Microbiologist OSD/NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Office of Scientific Director

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

B. pertussis produces various substances with striking biological activities. One of these substances is a protein that has been purified and crystallized by us and named pertussigen. It is a substance that in nanogram doses induces an increased immune response to many antigens; in particular it increases the production of IgE antibodies. It augments the intensity and duration of delayed type hypersensitivity (DTH), the induction of experimental allergic encephalomyelitis (EAE) and the production of insulin. It also induces lymphocytosis and increases the number of plasma cell precursors in the bone marrow of mice. Pertussigen has a molecular weight of around 100,000 and it is composed of 5 peptide chains. The role of the 5 peptides on the various activities of pertussigen is not well known, and this is under investigation. In addition, pertussigen is an excellent adjuvant to certain vaccines and this aspect is also under investigation in collaboration with others.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00378-01 OSD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Attempted Isolation of AIDS Agent from High Risk Specimens

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Robert M. L. Buller, Visiting Associate, OSD, NIAID

COOPERATING UNITS (if any)

Dr. Wyatt, LID, NIAID; Dr. Bloom, LPVD, NIAID; Dr. Hoggan, LVD, NIAID

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Office of the Scientific Director

SECTION

Office of the Scientific Director

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL MANYEARS: PROFESSIONAL: OTHER:

.2

.1

.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The etiology of the underlying immune deficiencies seen in acquired immune deficiency syndrome (AIDS) is unknown. Current observations suggest that a transmissible agent, possibly a virus, may be involved.

A parvo-like virus has been suggested as the possible etiological agent for AIDS because a number of characteristics of AIDS are exhibited in the pathogenesis of individual members of the parvovirus family.

Successful isolation of the AIDS agent will be difficult due to the large number of opportunistic infections present in AIDS patients. Therefore, it is essential to have access to clinical specimens obtained early in the course of AIDS. Commencing in the autumn of 1983, a large number of such samples collected prospectively by an NIAID intramural contract from several male homosexual populations in New York will be available for analysis.

We propose to attempt to isolate the AIDS agent from specimens of semen, feces, and lympho-reticular cells. The minimum number of cell lines needed to support the widest range of viruses including defective and non-defective parvoviruses will be utilized in the isolation procedure. These lines will include human, simian, rodent, canine, and feline cells. Dual infection with species-specific adenoviruses and (where feasible) herpes viruses will also be carried out for possible induction of adenovirus-associated-like agent growth. Since the agent may exhibit limited or no cytopathic effect in tissue culture, and may replicate inefficiently or in an abortive fashion, the cultures will also be scored by IFA using pre, acute and convalescent sera from AIDS patients as the "driving" antibody, as well as a panel of known positive virus antisera.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00383-01 OSD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Acquisition of Specimens from Cases of Acquired Immune Deficiency Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Richard G. Wyatt, M.D., Medical Officer, OSD/NIAID

COOPERATING UNITS (if any)

Dr. Louis Baker, New York Blood Center and Memorial Sloan Kettering Cancer Center
and Dr. Jonathan Gold, New York Blood Center and Memorial Sloan Kettering
Cancer Center

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SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The etiology of Acquired Immune Deficiency Syndrome (AIDS) is not known, although currently a variety of microbial agents has been proposed as playing a causative role. Since opportunistic infections with a variety of such viral, bacterial, fungal and parasitic agents occurs characteristically in AIDS, the question of whether an agent is etiologic or opportunistic arises frequently. The recovery of an infectious agent(s) or the demonstration of an antibody response to such an agent prior to the diagnosis of AIDS would provide evidence for primary infection with a putative etiological agent.

The major obstacle to efforts directed at cultivation or serological characterization of putative etiological agents has been the availability of early specimens obtained prior to the onset of AIDS. These specimens would be most likely to contain the agent, and even earlier sera would be required to represent pre-exposure sera.

In response to these needs, an intramural research contract was developed with the New York Blood Center and Memorial Sloan Kettering Cancer Center to collect specimens in a prospective fashion from 325 homosexual males. Three populations are being recruited: patients with lymphadenopathy (100), normal plasma donors (175) and normal homosexual males from a geographically distinct area in New York State (50). Specimens of peripheral blood leukocytes, plasma, serum, semen, urine, saliva and stool will be collected at 1 to 4 month intervals along with epidemiological information. Specimens will be stored in an NIAID Repository until cases of AIDS are identified in the study population. At that time, appropriate specimens will be studied intensively for the presence of agents, and paired sera will be examined for the development of antibody to various candidate agents.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00384-01 OSD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evaluation of Immunologic Abnormalities in AIDS Patients

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Thomas Folks, Ph.D., Staff Fellow, LIG, NIAID

COOPERATING UNITS (If any) Dr. Anthony Fauci, LIR/NIAID; Dr. Clifford Lane, LIR/NIAID; Dr. Steven Straus, LCI/NIAID; Dr. Louis Baker, NYBC; Dr. Jonathan Gold, Memorial Sloan Kettering; Dr. Donald Armstrong, Memorial Sloan Kettering; Dr. Robert Redfield, Walter Reed Army Hospital

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INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

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3.1 1.5 1.6

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- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies are being conducted to evaluate the effects of sera and plasma from AIDS patients on the growth and responsiveness of T cell lines and clones. Mixed leukocyte culture assays are also being used as a system in which to study suppressor factors from materials. As much as 70 percent suppression has been found from pooled AIDS plasma on the MLC response. Experiments are underway to determine any functional changes in B cell activation following cryopreservation of AIDS lymphocytes. Macrophage and null cell antibody directed cell mediated cytotoxicity are also being studied. Macrophage cultures have been established for the purpose of propagating and studying any potential AIDS agents.

Biochemical and functional characterization of immune-complexes from AIDS patients sera are currently underway. Isolation of these complexes are being evaluated for viral content by DNA blotting and suppressor effects in the MLC assay. Other serological markers from AIDS patients which are currently being evaluated are B₂ microglobulin and changes in isotype levels.

Recently, helper to suppressor ratios of AIDS patients have been performed (for investigators in the Institute) using the FACS-Analyzer. In addition to routine screening, we are establishing normal parameters for use of the analyzer and methods for determining phenotypes of lymphocytes from normals and AIDS patients following cryopreservation. Using the FACS-II, two color parameter analysis, studies of normal and AIDS lymphocytes has also begun.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00385-01 OSD

PERIOD COVERED

October 1, 1982 to September 30, 1983

(Title of project (80 characters or less. Title must fit on one line between the borders.)

Immunologic Evaluation of Chimps Exposed to Materials from AIDS Patients

*(Principal Investigator (List other professional personnel on subsequent pages.)**(Name, title, laboratory, and Institute affiliation)*

Thomas Folks, Ph.D., Staff Fellow, LIG/NIAID

(Cooperating units (if any))

Dr. Robert Purcell, LID/NIAID

LAB/BRANCH

Office of the Scientific Director

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

1.2

PROFESSIONAL:

0.3

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

(Summary of work (Use standard unreduced type. Do not exceed the space provided.)

A colony of chimpanzees currently housed at Meloy Labs are being evaluated for a number of viral hepatitis studies. In addition to these studies, a number of chimpanzees have received material from AIDS patients and are currently being followed in an attempt to transmit AIDS to non-human primates. Routine weekly helper to suppressor ratios (H:S) have been carried out. As of this date, no major changes have been found (five months). We have found that chimps have a greater number of suppressor marked non-T cells than do humans (13.9 vs. 5.8 respectively). Functionally, these cells appear to be natural killer cells. H:S ratio changes have been observed in hepatitis B virus (HBV) infected chimps at times of HBV inoculation and in NANB inoculated chimps prior to peak enzyme level detection. B cell activation assays are also being studied as a marker for early AIDS detection, along with B₂ microglobulin and HTLV serology.

LABORATORY OF BIOLOGY OF VIRUSES

1983 Annual Report

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LABORATORY OF BIOLOGY OF VIRUSES

National Institute of Allergy and Infectious Diseases

SUMMARY - October 1, 1982 - September 30, 1983

The past several years have seen a significant change in the focus of work within the Laboratory of Biology of Viruses. Recombinant DNA technology and DNA sequencing techniques have permitted the examination of conceptionally new problems. For most of the small viruses, precise knowledge of virion structure has been available but recombinant methodology has allowed detailed analysis of regions of the larger viruses to be defined. Using data obtained by DNA sequencing, information on viral specified RNA can now be compared with the DNA template from which it was copied. This has provided an understanding of how simple genomes can generate multiple viral proteins by using alternate splice sites to form overlapping but unique messenger RNA molecules. The precise localization of the start sites for transcription of specific genes has permitted the identification of regions that lie upstream from the start sites which regulate the rate of transcription. The localization of these control regions has allowed investigators to enhance or suppress expression of specific genes by introducing site specific mutations into these control regions. The insertion of foreign genes in the vaccinia virus genome which is described in this year's annual report offers an exciting new approach to the development of live vaccines. Hepatitis B surface antigen is actively expressed when the gene coding for that function is inserted into a non-essential region of the vaccinia genome, and virus containing this recombinant DNA genome is used to vaccinate rabbits.

A major goal of modern biology has been to define the relationship between the information contained within the DNA and its functional expression by a virus or a cell. Projects described in this year's annual report demonstrate that we are achieving that goal.

Laboratory of Biology of Viruses
National Institute of Allergy and Infectious Diseases

SUMMARY - October 1, 1982 - September 30, 1982

HONORS AND AWARDS

Dr. Norman P. Salzman continued to serve on the Editorial Board of the Journal of Virology, the Editorial Advisory Board, Biochemistry, the Scientific Board of the Coordinating Council for Cancer Research, and the NIAID Promotion and Tenure Committee. He served as Professorial Lecturer, Georgetown University School of Medicine. He participated in the U.S.-French Cancer Exchange Program in the summer, 1983.

Dr. Bernard Moss was Chairman of Animal Cells and Viruses, Gordon Conference 1983. He also served on the Advisory Committee on Nucleic Acids and Protein Synthesis, American Cancer Society. He was the Editor, Journal of Biological Chemistry and was on the Editorial Boards of the Journal of Virology, Virology, Antimicrobial Agents and Chemotherapy, and Intervirology.

Dr. Mark Cochran was awarded a postdoctoral fellowship from the National Sciences and Engineering Research Council of Canada.

Dr. James Rose was appointed chairman of the NIAID personnel Advisory Board.

Some of the highlights of this year's research efforts are detailed below.

PAPOVAVIRUSES

A. The SV40 Major Promoter is Controlled by Two Upstream DNA Domains that are Required for Efficient *In Vitro* Transcription

Previously, an 11 base DNA sequence 5'-G-G-T-A-C-C-T-A-A-C-C-3' (SV40 map position 294-304) which is important in the control of SV40 late RNA expression *in vitro* and *in vivo* had been observed. A second important domain of the SV40 late promoter has been identified. A series of mutants with deletions extending from map position 0 to 300 were prepared by Bal31 nuclease treatment. The cloned templates were then analyzed for efficiency and accuracy of late SV40 RNA expression in the Manley *in vitro* transcription system. Our studies show that in addition to the promoter domain near map position 300, there are essential DNA sequences between nucleotide position 74 and 95 that are required for efficient expression of late SV40 RNA. Included in this SV40 DNA sequence are two of the six GGGCGG SV40 repeat sequences and an 11 nucleotide segment which shows strong homology with the upstream sequences required for the efficient *in vitro* and *in vivo* expression of the histone H₂A gene. The position of this upstream promoter sequence can be moved 72 nucleotides closer to the major late cap site without any effect on the efficiency or accuracy of transcription. In vitro promoter competition analysis demonstrates that the upstream promoter sequence, independent of the 294-304 promoter element, is capable of binding polymerase/transcription factors required for SV40 late gene transcription. While two upstream domains control the extent of transcription, DNA sequences which control the specificity of RNA initiation at nucleotide 325 lie downstream of map position 294.

Studies have continued to define the molecular architecture of the domain located at base positions 294-304. Using a procedure of base misincorporation, a series of eleven KpnI resistant mutants have been obtained. The DNA sequences of each of the mutants and their transcription properties are currently being examined. (N. P. Salzman, Brady, Das, Nandi)

B. Improved Procedures for Directed Mutations in Regulatory Control Regions.

In the initial studies to generate site specific mutations, single-stranded regions were formed in the SV40 molecule at selected regions, and cytosines in these regions were deaminated using sodium bisulfite. Mutated viruses with altered properties could be generated by this procedure. However, the technique is of restricted value since only one of four deoxynucleotides can be modified and the technique also requires a restriction enzyme cleavage site at the region to be examined. Using chemical synthesis of deoxyoligonucleotides 15 to 20 bases long, it is possible to introduce mutations in any one of the four bases into DNA at any desired site in a molecule. These deoxyoligonucleotides are annealed to specific regions of the SV40 genome that have been inserted into a single-stranded bacteriophage. Using this procedure, we have generated additional mutations into the region that controls the SV40 late promoter. In this same region, the DNA sequences suggest that a hairpin structure can form and that it may serve a role in defining the 5' start site for transcription.

We have constructed a series of deoxyoligonucleotides that will either strengthen or disrupt this structure. These fragments are presently being incorporated into the SV40 molecules. In addition to these procedures for directed mutagenesis, we have also generated a series of related mutations at selected sites using the procedure of base misincorporation. With this latter procedure, a number of mutations have been generated in the third T-antigen binding site. (N. P. Salzman, Brady, Das, Nandi, Rodi, and Strike)

C. Purified RNA Polymerase II Recognizes Specific Nucleotide Sequences in the Adenovirus Promoter.

The properties of RNA polymerase II have been compared when it is purified or when the enzymatic acitivity is contained in the whole cell extract described by Manley *et al.* In the whole cell extract, transcription is initiated at the same 5' start sites that are used *in vivo*.

A major initiation site of purified calf thymus RNA polymerase II has been mapped on the cloned Adenovirus 2 major late promoter (Ad2 MLP). The 5' terminus of the major transcript in the TATA box is located 25 to 30 nucleotides upstream of the Ad2 MLP. The specificity of this initiation site has been determined by "runoff" transcriptional analysis and by RNase T1 analysis which employs single-stranded M13 phage DNA containing the Ad2 MLP as probe. This transcriptional initiation site is used when transcription was carried out on either a superhelical (FI) or linear (FIII) DNA template. Selective initiation of transcription on FI DNA could only be detected at 90, 120, and 150 mM $(\text{NH}_4)_2\text{SO}_4$ concentrations. In contrast, selective initiation of transcription on FIII DNA could be detected, by RNase T1 analysis, at $(\text{NH}_4)_2\text{SO}_4$ concentrations that ranged from 30 mM up to 120 mM.

These findings indicate that the purified enzyme enters into a complex with the DNA and migrates to the TATA or binds directly to the TATA box where it initiates transcription. In the presence of other protein factors, it initiates transcription 25-30 nucleotides downstream from this site. (N. P. Salzman, Mishoe, Vonsover, and Brady)

D. Enhanced in Vitro Expression of SV40 and Adenovirus Promoters

The whole cell (Manley) extract is able to carry out transcription *in vitro*, and initiation of transcription occurs at the same 5' start sites that are used *in vivo*. However, this system does not reflect the level of promoter utilization that is observed *in vivo*, and some promoters that function in the cell are not expressed *in vitro*. We have defined conditions which regulate transcription of the two SV40 late promoters. By altering the ionic conditions of the reaction, there is preferential expression of the promoter that initiates transcription at base position 325. Under these modified conditions, expression of the adenovirus IVa2 gene is readily observed *in vitro*, while under standard conditions, it is not transcribed. This adeno gene, like the SV40 late genes, lacks a TATA box upstream from the 5' start site. By constructing a series of upstream deletion mutations, an upstream domain has been identified that controls this gene. (N. P. Salzman, Natarajan)

PARVOVIRUSES

A. Synthesis of Adeno-associated Virus DNA Has Been Studied Using In Vitro Systems

The overall scheme of AAV DNA synthesis *in vivo* was first described by Straus *et al.* in 1976 (Proc. Natl. Acad. Sci. 73, p. 742). Briefly, following coinfection of KB cells with AAV and a helper Ad, AAV DNA synthesis is initiated on single-stranded genomic templates by a self-priming mechanism. Subsequent elongation yields a unit length hairpin intermediate. A second round of self-primed synthesis displaces the 5'-ended arm of the hairpin and leads to either (i) displacement of a complete plus or minus progeny strand (by virtue of a processing/synthesis step at the closed end of the hairpin) or (ii) concatemeric molecules if closed end processing/synthesis does not occur. These latter molecules can be eventually processed to unit length templates which, in turn, may also yield progeny strands by new self-primed rounds of displacement synthesis. It has been suggested that a similar synthetic mechanism may be involved in the replication of cellular DNA. At present, the specific enzymatic and regulatory factors (both cellular and viral) that participate in AAV DNA synthesis are not clearly defined. To help identify and characterize these elements, two *in vitro* DNA synthesizing systems have been utilized that generate AAV DNA replicating forms which correspond to those found *in vivo*. The systems are derived from nuclear extracts of Ad-AAV-infected, Ad-infected or uninfected KB cells. One system requires endogenous AAV DNA templates, the other requires exogenously added, denatured AAV virion DNA. Nuclear extracts are separated into two fractions, a sedimentation fraction (template fraction if derived from Ad-AAV-infected cells) and a supernatant fraction (polymerase fraction). Both fractions are required for synthesis on endogenous templates, whereas the exogenous template system utilizes only the supernatant fraction. Various specific requirements (e.g., ionic and polymerase) as well as structural features of DNA products have now been well characterized. Two distinct and potent inhibitors of DNA synthesis on single-stranded AAV templates have been extensively purified from KB cells. (Rose, Wang, Sebring and Ohi)

B. Cloned Genes of Adenoviruses are Required for Translation of AAV.

Several early adenovirus (Ad) gene products are required for replication of defective parvoviruses (AAV) (Janik *et al.*, 1981, PNAS 78, 1925). Definition of specific helper functions of these factors would provide insight into biochemical details of AAV replication, and conversely, could help to determine their individual roles in the regulation of Ad macromolecular synthesis. To investigate AAV transcriptional and translational requirements for Ad gene products, a duplex AAV DNA segment (0.03-0.97 map units) was cloned in pBR325 (pLHI). When 293-31 cells were transfected with this plasmid, transcripts were produced which were capable of directing *in vitro* synthesis of the three AAV structural polypeptides, although *in vivo* synthesis of AAV polypeptides could not be detected by immuno-fluorescence or immunoprecipitation. Cotransfection with pLHI and intact Ad DNA enhanced the level of cytoplasmic AAV transcripts, and *in vivo* synthesis of AAV proteins was readily demonstrable. It was found, however, that combined transfection with pLHI and a recombinant plasmid that contained the Ad DNA-binding protein gene (pDBP) also enhanced the level of cytoplasmic AAV transcripts but did not promote *in vivo* AAV protein synthesis.

When another plasmid that contained the Ad VAI RNA gene (p2VA) was cotransfected with pLHI and pDBP, in vivo AAV polypeptide synthesis occurred. pLHI plus p2VA, however, did not induce in vivo AAV protein synthesis. We conclude that Ad DNA-binding protein augments the level of AAV cytoplasmic transcripts, whereas translation of these transcripts requires Ad VAI RNA. Furthermore, based on these and other experiments, it appears likely that DNA-binding protein also plays a role in the translation of viral mRNA, and that this activity may require a direct interaction with VAI RNA. These findings indicate the existence of a mechanism which regulates the expression of certain viral proteins with a high degree of specificity. In all probability, a counterpart(s) of this mechanism operates in normal cells, providing an analogous capability for regulating synthesis of particular cellular proteins. (Rose, Koczot)

C. The Coding Sequences of AAV that Specify the Three Structural Proteins in the Virion Are Being Mapped.

It had previously been demonstrated that adeno-associated virus (AAV) contains three structural proteins designated A (~90,000 daltons), B (~75,000 daltons), and C (~60,000 daltons). The composition in virions, which also reflects in vivo synthetic rates, is roughly 90% C with comparable amounts of A and B (about 5% each). Current models of AAV protein synthesis dictate that a single message generates these proteins. However, its entire coding capacity from the site of transcription initiation (taking into account an intervening sequence) is barely long enough to code for a protein the size of A alone. Since the intervening sequence is proximal to the 5'-most AUG (initiation codon) in the body of the mRNA, the three proteins probably do not arise through differential splicing. In addition, our accumulated data do not clearly support a post-translational processing mechanism. Thus, it is problematic how three distinct proteins can arise by translation from a single mRNA species. To further explore various possibilities, we investigated structural homologies among these proteins.

First, it was found that slight differences exist between the corresponding proteins of AAV1 and AAV2. Proteins A and C of AAV1 are slightly smaller than A and C respectively while B of AAV1 is slightly larger than B of AAV2. In addition, supernumerary bands of C (i.e., four distinct subspecies of C) are present in both AAV1 and AAV2. These findings indicate some common control feature in the genomes of both AAV1 and AAV2 which results in very similar overall protein structure although there is little or no antigenic relatedness between AAV1 and AAV2 proteins. Secondly, staphylococcal protease V8 digestion of AAV2 proteins yielded at least ten cleavage products ranging in size from 8,000 daltons through 20,000 daltons which were identical in peptides A, B, and C. There were two bands that appeared in the digestion of C but not A or B. There was a band that appeared in A and C but not in B. These findings indicate that all three proteins, A, B, and C, are translated in the same reading frame, and that their overlap most likely encompasses all of C. We have now demonstrated that these AAV proteins arise by independent initiations and not, as previously thought, by proteolytic processing of a primary protein. (Rose, Sebring and Becerra)

D. Viral Specified RNAs and Proteins Synthesized by Kilham Rat Virus Have Been Characterized.

Five functional KRV specific mRNAs have been obtained from polysomes in infected cells. The longest KRV RNA is 4.7 kilobases (kb) and represents a transcript of 95 to 100% of the viral genome. The most abundant KRV-RNA is 3.0 kb representing about 50% of the viral genome and probably codes in the reticulocyte transcribing system for the most abundant viral protein (m.w. 68,000). It has been mapped on the viral genome and its origin of transcription has been determined.

Since the KRV genome contains approximately 5,000 nucleotides, the maximal protein coding capacity is for protein(s) with a total maximal molecular weight of 187,000. The KRV capsid proteins with molecular weights of A (89,000) and B (65,000) would require the coding capacity of almost the entire genome. Since nonstructural viral proteins are also present in the infected cell, the coding capacity of the viral genome must involve the use of some type of overlapping gene sequences. Using peptide analysis it has been shown that the capsid proteins are translated in the same reading frame from overlapping sections of the genome. The nine peptides found in fingerprinting protein B are all identical to nine of the 12 peptides found in the protein A fingerprint. Thus, there is either more than one promoter in the DNA sequence translated or the larger protein is processed after transcription. (L. A. Salzman, Mitra and Brown)

ADENOVIRUSES

A. Unique Terminal Structures Exist in Adenovirus 18 DNA.

It was previously demonstrated that DNA molecules extracted from adenovirus (Ad) particles (linear duplexes with molecular weights of $20-25 \times 10^6$ daltons) contain an inverted terminal repetition (ITR). The molecular arrangement of the repetition was initially determined by direct visualization of "panhandle" projections on circularized single-stranded Ad18 DNA. More recently, others have analyzed ITRs in the genomes of Ad types 2, 3, 5, 7, and 12 by nucleotide sequencing. For the major infectious components of encapsidated DNA, the lengths of these repeats have been found to vary from 103 bases (Ad2) to 162 bases (Ad12). In the case of Ad18, however, we noted previously that DNA molecules recovered from the main band of CsCl-purified virions possessed occasional ITR variability, with some repetitions approaching 20% of genome length. Furthermore, we found that increases in ITR length were accompanied by equivalent inward deletions of adjoining sequences, so that total molecular length remained nearly constant. Although it is probable that molecules with inward deletions are defective, it is not known for certain whether any molecule with an ITR is actually infectious (e.g., the repeat is always associated with a critical, small inward deletion), or, if infectious genomes do possess an ITR, whether ITR length can vary. To answer these questions, AD18 DNA from two plaque isolates (P-1 and P-2) derived from the prototype strain was analyzed by cleavage with restriction endonucleases and by electron microscopic heteroduplexing techniques. Both the P-1 and P-2 ITR genotypes remained unchanged after five additional virus passages. These findings indicate that infectious Ad18 genomes can carry ITRs of different length. Based on direct nucleotide sequence analysis, the P-1 ITR is 165 bases long and possesses

extensive homology with the ITR of Ad12. In addition, the P-1 ITR contains several short base tracts that are variably present in the ITRs of more distantly related serotypes. One of these tracts (5'...TGACGT) is also found near the ends of DNA from both autonomous and Ad-dependent (AAV) parvoviruses. (Rose, Garon and Ohi)

POXVIRUSES

A. Regulation of Vaccinia Virus Gene Expression

1. Transcriptional mapping of the vaccinia virus thymidine kinase and neighboring genes. Previously, we used marker rescue techniques to map the thymidine kinase gene within the HindIII J fragment of vaccinia virus. Further studies indicated that enzymatically active thymidine kinase (TK) was made in reticulocyte lysates programmed with early vaccinia mRNA that hybridized to plasmid recombinants containing either of two adjacent small DNA subsegments of the viral HindIII J fragment. The map position of an early polypeptide, with a molecular weight of 19,000 (19K), coincided precisely with that of the TK. The absence of the 19K polypeptide in cell-free translation products of hybridization-selected mRNAs from several TK-negative mutants provided an independent identification of the TK polypeptide. The small size of the TK polypeptide of vaccinia virus distinguishes it from that of procytotes, eucaryotes, and herpesvirus. Five early mRNAs of 3,840, 2,390, 1,790, 1,070, and 590 nucleotides were mapped within the HindIII J fragment by RNA blotting and nuclease S1 digestion of RNA-DNA hybrids. The RNAs of 590 and 2,380 nucleotides were found to have 5' coterminal ends and represent major and minor forms, respectively, of the TK message. The 3' end of the minor TK mRNA appeared to be coterminal with the 3' end of the 1,790-nucleotide transcript which encodes a 41K polypeptide. The 1,070-nucleotide RNA was identified as the message for a 21K polypeptide. All of these RNAs, including the two forms of the TK message, were made by the putative TK-negative nonsense mutants.

2. Nucleotide sequence of the vaccinia virus thymidine kinase gene and the nature of spontaneous frameshift mutations. Nucleotide sequencing of a 1,300-base-pair vaccinia virus DNA segment shown to contain a thymidine kinase (TK) gene revealed an uninterrupted reading frame of 177 codons capable of producing a polypeptide with a molecular weight of 20,102. Mapping of the TK mRNA by primer extension indicated a unique 5' end that precedes the initiation codon by only six nucleotides. Multiple 3' ends within a 10-nucleotide region, about 30 nucleotides beyond the termination codon, were located by nuclease digestion of DNA-RNA hybrids, and the length of the TK transcript, exclusive of polyadenylate, was estimated to be approximately 570 nucleotides. The region preceding the TK mRNA start site is extremely A+T rich and has sequence homologies with three other early genes. Genetic information is so compressed in this region of the DNA that the putative transcriptional regulatory sequence of the TK gene overlaps the coding sequence of a late gene. Only nine nucleotides separate the termination codon of the late gene from the initiation codon of the TK gene. Downstream, 66 nucleotides separate the TK termination codon from the apparent initiation codon of another early gene. The nature of three independent TK⁻ mutants was revealed by nucleotide sequencing. Each has a nucleotide reiteration leading to a +1 frameshift and a nonsense codon

downstream. The location of one frameshift mutation provided evidence that the first ATG is used for initiation of protein synthesis.

3. Development of a transient assay system for analysis of vaccinia virus gene expression.

Plasmids have been constructed that contain putative transcriptional regulatory sequences of vaccinia virus adjacent to the coding sequence for the bacterial enzyme chloramphenicol acetyltransferase (CAT). These plasmids express CAT after calcium phosphate mediated transfection in vaccinia virus infected cells but not in uninfected cells. This system is being used to define more specifically which sequences are involved in vaccinia virus promoter function.

4. Development of an in vitro system for expression of vaccinia virus genes.

Extracts from vaccinia virus infected cells have been shown to faithfully initiate transcription of vaccinia virus genes. Transcription is resistant to α -amanitin indicating that the viral RNA polymerase is involved. This system will be used to both study transcriptional regulatory sequences and to dissect out the protein factors needed for RNA synthesis.

(Moss, Weir, Haffey, Puckett and Cochran)

B. Structure and Replication of Poxvirus Genomes

1. Concatemeric forms of vaccinia DNA.

Previous studies from our Laboratory demonstrated that the ends of mature vaccinia virus DNA molecules are hairpin loops that are A+T-rich, incompletely base-paired, and exist in isomeric forms that are inverted and complementary (flip-flopped). To characterize the ends of replicating molecules, DNA was isolated from the cytoplasm at various times after virus inoculation and digested with appropriate restriction endonucleases. After agarose gel electrophoresis, the DNA fragments were transferred to nitrocellulose and probed by hybridization with ^{32}P -labeled vaccinia virus DNA. Autoradiographs revealed the presence of fragments containing terminal sequences that were twice the length of mature termini. These palindromes were present in DNA molecules that sedimented faster than unit length. Thus, we have evidence for formation of head to head concatemers during vaccinia virus replication. The hairpin loops at the ends of mature vaccinia virus DNA provide a mechanism for replicating the ends of a linear DNA molecule. Models that account for incomplete base-pairing and flip-flop sequence inversions of the hairpin loops as well as concatemer formation have been described.

2. Mapping the vaccinia virus DNA polymerase gene.

The resistance to phosphonoacetate (PAA) of a DNA polymerase isolated from cells infected with a PAA-resistant (R) mutant of vaccinia virus provided genetic evidence that the enzyme is virus coded. Furthermore, PAA resistance could be used as a dominant marker for mapping the DNA polymerase gene in a manner previously used in this laboratory to locate the thymidine kinase gene.

Initial experiments indicated that drug resistant recombinants could be isolated from cells infected with wild-type PAA-sensitive (PAA^R) virus following transfection with intact or HindIII digested DNA from PAA^R mutants. To locate the PAA^R marker, HindIII restriction fragments of PAA^R DNA were cloned in plasmid and cosmid vectors. Of these, only HindIII E exhibited marker rescue. By successive subcloning, the PAA^R marker was localized to a 2,000 bp DNA fragment about 57 kb from the left end of the genome.

(Moss, Jones)

C. Vaccinia Virus: An Infectious, Selectable, Eukaryotic Cloning Vector

1. Construction of insertion vectors. To facilitate the cloning and expression of foreign genes, special plasmid vectors were constructed. These plasmids contain the vaccinia virus thymidine kinase gene interrupted by insertion of a vaccinia virus promoter next to a polylinker containing multiple restriction endonuclease sites. Foreign coding sequences inserted into the latter sites will be under control of the vaccinia promoter. When cells infected with vaccinia virus are transfected with the insertion vector containing a foreign gene, homologous recombination occurs. This leads to insertion of the hybrid gene into the vaccinia virus thymidine kinase (TK) gene. Recombinants are TK⁻ and can be selected by plaque assay in the presence of bromodeoxyuridine.

2. Capacity of vaccinia virus for foreign DNA. To test the capacity of poxviruses for added foreign DNA, a recombinant was constructed that contains 24,700 base pairs of bacteriophage, λ DN A inserted within the vaccinia virus thymidine kinase gene. The recombinant is stable, infectious and replicates in tissue culture at the same rate and to the same titer as standard vaccinia virus. This size flexibility of the poxvirus genome and the lack of stringent packaging requirements are useful features for an infectious eukaryotic cloning vector.

3. Expression of hepatitis B virus surface antigen by infectious vaccinia virus recombinants. The coding sequence for hepatitis B virus surface antigen (HBsAg) has been inserted into the genome of vaccinia virus under control of vaccinia early promoters. Recombinants, selected by loss of thymidine kinase expression, are stable and retain infectivity. Cells infected with recombinant virus synthesize and excrete particles of HBsAg indistinguishable by antigenicity, polypeptide composition, buoyant density, sedimentation rate and size from particles present in the serum of humans infected with hepatitis B virus. Rabbits vaccinated with the recombinant virus rapidly produce antibodies against HBsAg, suggesting a potential use as a live vaccine in man.

(Moss, Mackett and Smith)

D. Vaccinia Mutants that are Resistant to Aphidicolin Have Been Isolated

The drug aphidicolin inhibits DNA synthesis in uninfected cells by interaction with DNA polymerase α . The DNA polymerase coded by vaccinia virus is also inhibited by the drug, but it is less sensitive than DNA polymerase α .

Viral DNA synthesis that is blocked by aphidicolin is returned to the normal rate by simply washing and suspending infected cells in drug-free medium. A mutant vaccinia virus which is resistant to aphidicolin has been developed. The mutant will produce substantial amounts of progeny in the presence of 120 μ M drug, the highest concentration tested. In vitro tests of viral DNA polymerase isolated from cells infected with wild-type and resistant virus indicate that the enzyme from the mutant is more resistant to the drug than the polymerase from wild-type virus. The aphidicolin resistant mutant is not resistant to phosphonoacetate which also interacts with the viral DNA polymerase.

(DeFilippes)

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00123-17 LBV

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Replication of Poxvirus Genomes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Bernard Moss Medical Director LBV, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: PROFESSIONAL:

2.9

1.3

OTHER:

1.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Poxviruses are large DNA viruses that replicate in the cytoplasm of infected cells. Vaccinia virus, the prototype for this group, has a 180,000 base pair (bp) linear double-stranded DNA genome with covalently linked ends and a 10,000 bp inverted terminal repetition. Nucleotide sequence analysis revealed that the ends of the genome consist of hairpin loops so that the two DNA strands form one continuous polynucleotide chain. The 104 nucleotide apex of the loop is A-T rich, incompletely base-paired and exists in two isomeric forms that are inverted and complementary in sequence (flip-flopped). During replication, head to head concatemers are formed. Models for the replication of the terminal segment of the poxvirus genome that involve site specific nicking and lead to flip-flop rearrangements and head to head dimerization have been proposed. Enzymes involved in DNA replication are encoded within the vaccinia virus genome. Using phosphonoacetate resistance as a genetic marker, the DNA polymerase gene has been mapped within a 2,000 bp DNA segment about 57 kb from the left end of the genome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00126-10 LBV

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional Analysis of Vaccinia Virus DNA

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Frank DeFilippes, Ph.D.

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The drug aphidicolin inhibits the reproduction of vaccinia virus. DNA synthesis in uninfected cells is reduced by the interaction of aphidicolin with DNA polymerase α . The DNA polymerase coded by vaccinia virus is inhibited by the drug, but it is less sensitive than DNA polymerase α . At drug concentrations greater than 40 μM both plaque formation and viral yield show a rapid exponential decrease. At 20 μM there is only a slight decrease in the number of plaques, but they are reduced in size. Introduction of the drug 2 hr prior to infection, at infection, or 1.5 hr after infection does not significantly alter the inhibition of viral yield. Also, the effect of the drug on viral yield is completely reversible for at least 12 hr after infection.

Aphidicolin reduces virus formation by reducing the rate of viral DNA synthesis. With 20 μM drug present for 6 hr after infection, viral DNA synthesis is reduced to 12% of control values. Viral DNA synthesis is returned to the normal rate by simply washing and suspending infected cells with drug-free medium.

A mutant vaccinia virus which is resistant to aphidicolin has been developed. The mutant will produce substantial amounts of progeny in the presence of 120 μM drug, the highest concentration tested. In vitro tests of viral DNA polymerase isolated from cells infected with wild-type and resistant virus indicate that the enzyme from the mutant is more resistant to the drug than the polymerase from wild-type virus.

The aphidicolin resistant mutant is not resistant to phosphonoacetate which also interacts with the viral DNA polymerase.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00290-02 LBV

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Site Specific and Deletion Mutants which Regulate SV40 Transcription

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Norman P. Salzman, Ph.D., LBV, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

6.5

PROFESSIONAL:

4.4

OTHER:

2.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In most eucaryotic genes, a common set of nucleotides has been found before the start site of RNA transcription. These nucleotides, referred to as either a TATA or Goldberg-Hogness box, are important determinants of transcription in vivo and in vitro. The control region for the late SV40 transcripts have been examined by site specific mutagenesis and by generation of deletion mutations. Mutations that enhance or suppress the transcriptional activity of a single start site have been identified and differ from the consensus Goldberg-Hogness box. An additional cluster of nucleotides that is located at base positions 75-96 also control the level of transcription. It maintains this enhancing effect when its position relative to the 5' start site is altered.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00291-02 LBV

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interaction of RNA polymerase II with defined DNA templates

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Norman P. Salzman, Chief, LBV, NIAID

COOPERATING UNITS (if any)

Dr. Gerald Lancz, Department of Medical Microbiology, University of South Florida, Tampa, Florida

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.4

PROFESSIONAL:

2.4

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The interaction of purified calf thymus RNA polymerase II and SV40 or adenovirus DNA have been examined. The enzyme binds to the DNA at specific sequences, but these sequences are not identical to the 5' start sites used in vivo or in vitro in a system containing a crude cell free extract. The enzyme also binds to nicks in DNA and forms the transcript which is an RNA-DNA adduct. During chain growth, the enzyme pauses or terminates RNA chains at specific sites.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00292-02 LBV

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Factors Required for Specific Transcription

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Norman P. Salzman, Chief, LBV, NIAID

COOPERATING UNITS (if any)

None

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Laboratory of Biology of Viruses

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.2	1.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In vitro transcription systems are used to identify promoter signals and factors that control transcription in eucaryotic systems. These systems accurately initiate transcription from a wide variety of promoters. Preferential stimulation of certain promoters is achieved in the presence of low concentrations of ammonium sulfate. The addition of $(\text{NH}_4)_2\text{SO}_4$ allows detection of promoters which could not otherwise be detected.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00293-02 LBV

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Proteins Associated with the Parvovirus, KRV, and its DNA

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Lois A. Salzman, Ph.D. Research Chemist LBV, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.2	3.0	1.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The parvovirus, KRV, is a member of an increasingly important group of small eukaryotic viruses. We have been studying the replication of this virus in the infected cell. We have isolated the virus specific RNAs, characterized them and used them in an erythrocyte translating system to synthesize in vitro viral proteins. We have studied the relationship of the viral proteins by peptide finger printing. We are also studying the terminal protein covalently linked to the replicating viral DNA. We hope to determine its function in virus replication.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00294-02 LBV

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Adenovirus DNA

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

James A. Rose, M.D. Section Head LBV, NIAID

COOPERATING UNITS (if any)

R. Padmanabhan, University of Kansas School of Medicine, Kansas City, KS 66102

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.15

PROFESSIONAL:

.75

OTHER:

.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The current objective of these studies has been the application of physical and biochemical techniques to characterize the length and structure of inverted terminal repeats (ITR's) in the genomes of both oncogenic and nononcogenic human adenoviruses (Ad). Our ultimate goal is to define the roles of these sequences in regulating viral and cell growth (e.g., infectious yield and efficiency of cellular transformation). We have now described several important structural features of Ad ITRs (i.e., size, variations and homologies). Among methods used are gradient sedimentation, DNA cleavage with restriction endonucleases, gel electrophoresis, base sequence analysis and electron microscopy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00295-02 LBV

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Helper Factors Required for Expression of the Adeno-Associated Virus Genome

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

James A. Rose, M.D. Section Head LBV, NIAID

COOPERATING UNITS (if any)

None

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Laboratory of Biology of Viruses

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:

1.4

.2

1.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main objectives of this project are (i) to define where and how each required helper virus factor regulates expression of defective human parvovirus (AAV) genomes and (ii) to relate these findings to their respective roles in the replication of the helper viruses (adenoviruses, herpesviruses) themselves as well as to potentials for selective interference with viral infection. We previously mapped the adenovirus genes required for AAV replication and have recently found that two of these gene products, VAI RNA and the 72K DNA-binding protein, are required for translation of AAV mRNA. Among methods used are cleavage of DNA with restriction endonucleases, DNA cloning, gel electrophoresis, blot-hybridization analyses and DNA transfection of cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00296-02 LBV

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization and Production of Parvovirus Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

James Rose, M.D. Section Head LBV, NIAID

COOPERATING UNITS (if any)

(1) Richard McPherson, Georgetown University Hospital, 3800 Reservoir Road, N.W.
Washington, D. C. 20007

(2) Carl W. Anderson, Brookhaven National Laboratory, Upton, New York 11973

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Laboratory of Biology of Viruses

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.6	.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main objectives of these studies are (i) to identify and characterize all proteins that are specified by the defective human parvoviruses (AAV) and to determine similarities and differences with autonomous parvovirus proteins, (ii) to define the mechanism(s) by which the AAV proteins arise and (iii) to define specific functions of the AAV proteins. We have now found several AAV structural protein species previously not known to exist. Post-translational processing does not appear to account for production of any AAV structural proteins, although they share large proportions of sequences-in-common. The mechanism that regulates translation of AAV proteins is of fundamental interest and is now being investigated. Among method used are affinity chromatography, gel electrophoresis, in vitro translation of viral RNA, electrophoretic and HPLC analyses of V8 protease and tryptic peptides and aminoterminal sequencing of purified polypeptides.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00297-02 LBV

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism and Regulation of Adeno-Associated Virus DNA Replication

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

James Rose, M.D. Section Head LBV, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Molecular Structure Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.9

PROFESSIONAL:

2.1

OTHER:

.8

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary objective of this project is to define biochemical mechanisms involved in eukaryotic DNA synthesis. To approach this problem, we are investigating adeno-associated virus (AAV) and adenovirus DNA replication in in vitro systems. We have now shown that replicating forms of AAV DNA can be generated in vitro utilizing either endogenous or exogenously added templates and cellular polymerase, and that de novo initiation of DNA synthesis can occur in vitro. Two potent inhibitors of AAV DNA synthesis have been purified from KB cells. Among methods used are differential centrifugation, ion exchange and affinity chromatography, gel electrophoresis and isoelectric focusing.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00298-02

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vaccinia Virus: An Infectious, Selectable, Eukaryotic Cloning Vector

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Bernard Moss Medical Director LBV, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.75	2.4	1.35

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Vaccinia virus is a large DNA virus that replicates within the cytoplasm of eukaryotic cells. We have succeeded in developing this virus as a eukaryotic cloning vector with potential use as a live vaccine. The initial step was construction of plasmid recombinants containing foreign DNA flanked by vaccinia virus DNA sequences. The plasmid DNA was then introduced into cells infected with vaccinia virus where homologous recombination occurred. Selection of viral recombinants containing foreign DNA inserted into the vaccinia thymidine kinase gene was achieved by plaque assay in the presence of 5-bromodeoxyuridine. Expression of the foreign genes was dependent on vaccinia virus regulatory sequences. Restriction endonuclease analysis, agarose gel electrophoresis, and hybridization to ³²P-labeled DNA was used to confirm that site-specific recombination had occurred. Thus far, a number of foreign genes including herpesvirus thymidine kinase, prokaryotic chloramphenicol acetyltransferase, and hepatitis B virus surface antigen have been expressed. In the latter case, experimental animals vaccinated with the recombinant virus produced a specific immune response to the foreign antigens.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00307-02 LBV

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Vaccinia Virus Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Bernard Moss Medical Director LBV, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Biology of Viruses

SECTION

Macromolecular Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

4.45

PROFESSIONAL:

3.45

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Detailed transcriptional maps and translational maps of selected portions of the vaccinia virus genome were constructed. More than 16 early transcripts were characterized and six early genes were sequenced. These studies revealed that early mRNAs are colinear with the genome, unspliced, and have multiple closely spaced 5' ends. The nucleotide sequences for about 60 base pairs upstream of transcriptional initiation sites are extremely A+T rich and contain long runs of As and Ts. The eukaryotic A₂TA₃ poly(A) signal sequence was not found near the ends of the genes. Marker rescue and in vitro translation techniques were developed to map functional vaccinia virus genes. In this manner, thymidine kinase was mapped within HindIII J fragment and sequenced. The nature of 3 independent TK⁻ mutants was revealed by nucleotide sequencing. Each had a nucleotide reiteration leading to a +1 frameshift. In vitro and transient assay systems were developed to define regulatory nucleotide sequences and isolate regulatory proteins.

LABORATORY OF CLINICAL INVESTIGATION
1983 ANNUAL REPORT
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SUMMARY OF PROGRAM

Laboratory of Clinical Investigation
October 1, 1982 to September 30, 1983

Michael M. Frank, M.D., Chief of Laboratory
and Clinical Director, NIAID

INTRODUCTION

The year ending in October, 1983 has seen considerable growth and strengthening of the Laboratory of Clinical Investigation Program. Although no new groups have been formed during this fiscal year, many of the groups have been strengthened and the overall program is now in the general form that we would like to maintain. Currently there are three program areas within the Laboratory of Clinical Investigation. The Infectious Disease Program area is comprised of several sections. Dr. John Gallin and Dr. Bruce Seligmann study the phagocytic cell and its responses. They also study patients with phagocytic cell defects of many sorts. This group, concerned with the phagocytic cell, is joined by sections that focus on each of the general groups of microorganisms. Thus, Dr. Stephen Straus is interested in many aspects of clinical and basic virologic disease and his viral disease section has grown in stature during this year. We have recruited a new member of that group who will be joining the LCI as a Ph.D. basic virologist in the next fiscal year. Dr. John Bennett is concerned with fungal disease and June Kwon-Chung continues to be an important member of that group concerned with more basic aspects of fungal genetics and metabolism. Dr. Eric Brown and Dr. Keith Joiner, although members of the Clinical Immunology Section, continue to develop their interest in host defense against bacterial infection and in the process of phagocytosis from the point of view of complement and complement interactions with receptors. Dr. Thomas Quinn provides a focus for our investigative efforts in sexually transmitted diseases. We continue to have a close liaison with the Clinical Parasitology Program under Drs. Franklin Neva and Eric Ottesen with Dr. Ted Nash joining actively in the clinical aspects of our program. Patients with diseases referable to all of these various systems, including a wide variety of parasitic diseases, are admitted to the Clinical Center for study and therapy.

Our second major program area is the Clinical Immunology Group. The Clinical Immunology Section under Dr. Michael Frank, Clinical Director and Chief of the Laboratory of Clinical Investigation is interested in humoral aspects of immunity, the role of immune complexes in the development of disease, and the function of complement in the production of manifestations of disease. Members of the Clinical Immunology Section include Drs. Eric Brown and Keith Joiner as well as Dr. Carl Hammer and Ms. Thelma Gaither. A group under Dr. Warren Strober joined the Laboratory of Clinical Investigation last year and, during this fiscal year, has initiated extensive studies. The group is now completely equipped and able to begin both clinical and basic studies. Dr. Strober's group is the group interested in cellular aspects of immunity within the Laboratory of Clinical Investigation. Their particular interest is immunity across mucosal surfaces and defects in immunity across mucosal barriers. Their interests tend to focus on gastrointestinal disease with an interest in Crohn's disease, ulcerative colitis, gluten-sensitive enteropathy, etc. Dr. Stephen James is part of this group with a major interest in hepatic dysfunction, primary biliary cirrhosis, as well as

other aspects of gastrointestinal pathology.

The Allergy Program area continues to develop under Dr. Michael Kaliner. This program consists of Dr. Kaliner's group plus Dr. Dean Metcalfe and his collaborators. During the year, the program has been strengthened with the addition of strong junior personnel, including Dr. Thomas Keahay who is studying angioedemas and urticarias. The program studies many aspects of allergic disease ranging from asthma and allergic rhinitis on the one hand to mechanisms of hypersensitivity reactions on the other.

Thus, although the general format of the laboratory program has not changed, it has changed in depth with the addition of added resources. The number of associates in the program has grown from approximately five per year to seven per year. Although training of young physicians is not the primary goal of the Laboratory of Clinical Investigation, it has been found over the years that bright, aggressive, young physicians contribute enormously to the program by providing ideas and drive and that their training contributes to a national resource of considerable importance.

On April 1, 1982 an acute Infectious Disease research program within the Laboratory of Clinical Investigation was established under the direction of Dr. Quinn in the Division of Infectious Diseases at John Hopkins Hospital. The unit was established for the purpose of developing collaborative clinical research projects in the area of sexually transmitted diseases, and in the area of host defense mechanisms in acute infectious diseases. Research projects were established to provide an interaction between the intramural program and the program at Johns Hopkins Hospital. The research program is designed to facilitate direct access to patients with acute infectious diseases. As a result of this joint program between NIH and Johns Hopkins University Department of Medicine, rotation on the Infectious Disease Consultative Service is available and coordinated by Dr. Quinn for NIH clinical associates who desire further training in acute infectious diseases.

SUMMARY OF LABORATORY PROGRAMS

The Infectious Disease Group

The Bacterial Disease Section

This section has been concerned with many aspects of phagocytic cell function in normal states and in disease. The section has had a major interest in the way chemotactic factors that influence the directed migration of cells are stored in subcellular pools. They were the first to suggest that specific granules may contain chemotactic factor receptors that mobilize to the plasma membrane. The work in this area continues and has received great interest from the community of investigators who study phagocytic cell function. The group has also been interested in the role of ion flux in control of cell function and has raised the interesting possibility that high potassium concentrations found in pus may play an important regulatory role in determining the function of cells during the development of an abscess. The study of patients with neutrophil defects has been of particular interest. Patients with chronic granulomatous disease and patients with frequent infections and regulatory defects including individuals with the "Job's syndrome" have been studied. It was shown that the monocytes of some of these patients produce a product that inhibits granulocyte chemotaxis suggesting that this may contribute to infection. The group has proceeded much further in its attempts to fractionate neutrophils and neutrophil cellular elements. They are one of the early groups to use the new techniques available to produce neutrophil cytoplasts which contain plasma membrane and cytoplasm but few of the subcellular elements of neutrophils. These cytoplasts can phagocytose particles and, since they can be frozen, are an extremely important source of a simplified cell-like particles that can be evaluated to determine mechanisms of cellular function. This year studies were performed on the effect of pregnancy on neutrophil function. Interestingly, it was shown that there is defective production of superoxide ion in pregnant women suggesting that infection, sometimes seen during pregnancy, may in part result from neutrophil dysfunction. The group is exerting considerable effort to determine the basis of chronic granulomatous disease. They have performed a careful analysis of the genetics of this disease and have demonstrated that there are multiple modes of inheritance. Moreover, by mixing neutrophil cytoplasts from patients with chronic granulomatous disease and with different inheritance patterns, they have been able to correct the oxidative metabolism defect in vitro. This further indicates that these patients are heterogeneous with respect to their biochemical defects and suggests that it will be possible by analyzing the neutrophil cytoplasts to determine the specific biochemical defects responsible for the disease. The consequence of some of the defects has been further analyzed and has been shown that these patients, unlike normal individuals, do not turn off neutrophil migration into skin sites after the first wave of immigration as do normal individuals. Failure to control cellular immigration and activation may explain the propensity for granuloma formation in these patients. Practical questions concerning the best method for preparing neutrophils for infusion into patients with granulocyte defects who are infected have shown that the usual methods used by the Blood Bank for preparing neutrophils lead to a major loss of normal neutrophil function and have allowed for the development of new methods to prepare the neutrophils which allow for much greater functional activity. These studies of activation of leukocytes, mechanisms of cell adhesiveness, degranulation, and chemotaxis are of obvious importance in understanding the

normal process that takes place when a granulocyte participates in the host defense reaction. Ultimately, these studies will shed light on a wide variety of diseases in which neutrophil defects, congenital and acquired, play a role. Another important aspect of the work of this section has concerned the definition and study of neutrophil subsets. It was formerly thought that neutrophils were a homogeneous population of cells. It is now known that there are neutrophil subsets and that these neutrophil subsets appear to have different functional capacity. Whether one subset evolves into another is not known. In conjunction with collaborators, the group has developed monoclonal antibodies which recognize the various subsets, facilitating their identification and study. In another series of experiments, the group has studied a specific biochemical reaction within neutrophils called tyrosinolilation of a subcellular element, tubulin alpha chains. It was shown that patients with Chediak-Higashi syndrome and Chronic Granulomatous Disease have major abnormalities in this reaction. Additional studies have suggested that the redox state of the cell plays a major role in tubulin biochemistry. Further studies will allow us to determine how this contributes to neutrophil function and dysfunction in the normal and abnormal individual.

Viral Disease Section

This section has had major interest in the pathogenesis, molecular biology, immunology, natural history and therapy of human herpes virus infection. Both normal and immunocompromised patients with herpes infections have been identified, followed and treated. An important series of studies has been undertaken of the use of Acyclovir in the treatment and suppression of Herpes Simplex infections and it has been shown that this drug has real promise in this patient group. Studies of the molecular biology of the herpes virus genome have also continued and the Varicella Zoster virus genome has been completely mapped. Recombinant DNA clones have been developed which we hope will serve in the rapid diagnosis of Varicella and Herpes Zoster and as probes to determine the sites and nature of viral latency in human tissues. The group has also been involved in studies of the enteral adenoviruses. These viruses comprise what is now known to be two related sub serogroups of viruses that are a major cause of gastroenteritis in children. Dr. Straus' group is responsible for the current techniques for growing those viruses and have now been involved with mapping the genome and studying the differences in metabolism between these viruses and the more usual adenovirus strains. Molecular biological techniques have been used to develop sensitive assays for the enteral adenovirus genome in clinical materials. The group has also been deeply concerned with the recent AIDS epidemic that has occurred in homosexual males and other high risk groups. This is the Clinical Center group responsible for much of the current work on virus infection in these clinically ill patients and has studied the use of Acyclovir and other antiviral agents in this patient group. They have assisted all other studies of AIDS which have been ongoing. Our ongoing studies of Herpes virus infection in man and AIDS, emphasizes the interest that the Intramural Infectious Disease Program has in sexually transmitted disease.

During the last fiscal year the Johns Hopkins collaboration on sexually transmitted disease has been developed with Dr. Quinn providing access to patients with Herpes, with AIDS and Chlamydia infection. The NIH-Johns Hopkins Infectious Disease Research Laboratory has been primarily directed in specific research aspects of sexually transmitted diseases such as the sexual transmission

of enteric pathogens, the immunology and pathogenesis of Chlamydia trachomatis, and immunologic aspects of herpes simplex virus infection. A major commitment of this laboratory is to study the Acquired Immune Deficiency Syndrome (AIDS). The approach to AIDS is multifaceted and collaborative involving investigators at the National Institutes of Health, at Johns Hopkins School of Public Health and Hygiene, and the Johns Hopkins University School of Medicine. Areas presently under study are the clinical manifestations of AIDS with particular emphasis on the gastrointestinal presentation of AIDS, research on the possible viral etiology of AIDS with particular emphasis on parvovirus, immunologic alterations in patients with AIDS and lymphadenopathy with particular emphasis on immunosuppressive factors, alteration in the reticuloendothelial system in patients with AIDS, and the epidemiology and natural history of AIDS in homosexual men residing in Baltimore.

In addition to the research on AIDS, this laboratory continues its studies on the immunology and pathogenesis of Chlamydia trachomatis infections. This research encompasses both clinical aspects of Chlamydia trachomatis infection including cervicitis, salpingitis and proctitis. Animal models have been developed mimicking each of these diseases in non-human primates. The systemic and mucosal immune response of C. trachomatis is being carefully assessed in collaboration with Warren Strober with the use of recently developed monoclonal antibodies for C. trachomatis and for T-cell differentiation in mucosal lesions of tissue from infected patients and experimental animal infections.

In relationship to Dr. Quinn's longstanding interest in the gay bowel syndrome, the I.D. Research Laboratory has recently developed an ELISA for stool antigen detection for Giardia lamblia and Entamoeba histolytica. These assays are rapid, sensitive and specific for the diagnosis of these protozoans, and will be useful in epidemiologic surveys and in monitoring therapy. Present efforts within the laboratory are aimed at the development of an ELISA for detection of cryptosporidia antigen, a severe infection in AIDS patients. If successful, this assay can be utilized to define the epidemiology, natural history and spectrum of cryptosporidia infection.

Clinical Mycology Section

This group continues its careful analysis of the immune response of patients infected with cryptococci and who develop cryptococcal meningitis. These patients have been shown to be specifically non-responsive to cryptococcal antigen and the cause of the tolerance to cryptococcal polysaccharide is being explored. Characteristics of the normal response to cryptococcus are being determined in parallel with the response of patients with cryptococcal infection. The overall goal is characterizing the nature of the deficit seen in patients cured of systemic cryptococcosis. Understanding this defect may explain why they develop cryptococcal disease initially. Specific chemical studies of a capsule polysaccharide have also continued and the immunodominant portions of the polysaccharide that antibody recognizes have been determined. With regard to sugars available in the polysaccharide, computer modeling of the polysaccharide structure was performed, which suggested buried mannose groups within the molecule. The fact that these sugars are buried within the molecule explains the poor antibody response to them. An experimental model and mode of analysis were devised to study the effect of a drug, flucytosine, in treatment of this disease. It was of importance to determine the exact role of this drug in treatment of

patients with this disease. In this model, it was possible to show the beneficial effect of the drug. This had not previously been possible. Details of the metabolism of the two varieties of *Cryptococcus neoformans* have been explored in Dr. Kwon-Chung's group. Genetic and biochemical studies have previously shown that two varieties of this organism exist and biochemical tests have now been developed which show significant differences in the metabolism of these two varieties of organism. These metabolic differences have important consequences in terms of the way the organism may utilize various substrates in vivo and may ultimately have important therapeutic implications. A new variety of *Cladosporium* which had been originally reported, but then lost for study by earlier investigators, was re-identified and a new variety of *Cladosporium*, which had never been previously identified, was isolated from a brain lesion in man. Thus, the role of mycotic infections in the development of disease in man, continues to be explored. New organisms which cause human disease are being identified and metabolic characteristics which may make the development of antimicrobial agents possible are being identified.

Clinical Parasitology Section

The Clinical Parasitology Section works closely with the remainder of the Laboratory of Clinical Investigation staff in the study of immunologic and host defense aspects of parasitic disease. Much of the work of this section which also is part of the Laboratory of Parasitic Disease, is included with the summary of work of that laboratory. Nevertheless, it is of importance to mention that a wide variety of important studies have proceeded which involve a number of major parasitic diseases. Patients with a wide variety of these illnesses have been admitted to the Clinical Center for study of pathophysiology and treatment. A summary of the section's findings is included with LCI data. Detailed studies of lymphatic filariasis have been performed in an attempt to understand the immunoregulatory control of this important parasitic infection. The control of IgE in the development of this disease has been examined and work is being aimed at defining T-cell regulatory factors that regulate IgE synthesis. These patients may have very high levels of IgE antibody and hypersensitivity response to parasite antigens may be an important part of the pathophysiology of this disease. It has been shown that filariasis patients do not have the usual distribution of subclass IgG antibodies that normals have and the significance of this important new finding is under study. A complete analysis of the spectrum of antigenic specificity of the antibodies found in these patients is under development. The disease onchocerciasis is also under study. Under particular examination has been the severe side-effects that accompany the treatment of this infection, which limit the potential for mass chemotherapy of this disease. Patients were treated in Ghana and a variety of blood samples were taken back to the laboratory. In collaboration with the Clinical Immunology Section, it was shown that major abnormalities exist in the complement system following treatment, suggesting that this important mediator of inflammation may play a role in the development of these severe side-reactions. A study is also underway of chemoprophylaxis of *Loa Loa*. This is an important disease of normal Peace Corps volunteers entering Gabon, Cameroon and the Central African Republic. It is hoped to prevent the development of the disease in these volunteers. Studies are also underway of the pathophysiology of shistosomiasis and particularly of the immunosuppression that may occur in these patients in which they have poor or absent lymphocyte responses to parasite antigens. Moreover, their immunoglobulin, particularly IgE, rises. The immunoregulatory defect is being

examined in detail with an eye toward understanding the host defense mechanisms that are awry and the development of this disease which affects an enormous portion of the world's population. Tests have been developed for the identification of strongyloidiasis in patients and are currently being evaluated. Tests for subtypes of giardiasis are also undergoing an evaluation with an eye toward developing an understanding of the pathophysiology of infection and the control of infection in patient groups.

As discussed under the Clinical Immunology Section, two young investigators, Dr. Eric Brown and Dr. Keith Joiner have been examining the role of complement in protection against bacterial disease. These studies are reviewed under the section on Clinical Immunology.

Immunology Program

Clinical Immunology Section

The Clinical Immunology Section has continued to develop and expand its interest in two major areas; 1) the interaction of complement and complement components with bacteria and the consequences of that interaction and, 2) the role of receptors for complement fragments in phagocytosis and how these receptors function in normal physiologic mechanisms of the cell. To this end, considerable effort has been expended on purification and characterization of complement proteins, a difficult task. In the past year, Dr. Carl Hammer has refined his methods for the purification of these proteins and the purified proteins have been used to study a number of interactions. Particularly of note is the interaction of C5 and C6 in the so-called acid activation step. Here, a mixture of inactive C5 and C6, when acidified, generates a membrane attack complex. Dr. Hammer showed that in this reaction, C5 is cleaved even though there was no known C5 cleaving enzyme. It is of great interest that C6 has been thought by some to have a buried enzymatic site theoretically capable of proteolytic attack and the possibility is being actively explored that the C6, under acidified conditions, is activated and attacks C5 cleaving it to form a membrane attack complex. The role of this type of complex in the acid activation Ham's test used in diagnosis of paroxysmal nocturnal hemoglobinuria is under study. During this year, members of the section have also succeeded in developing a novel chromatographic technique for the purification of the C1 esterase inhibitor, a protein that has proven exceedingly difficult to purify in the past. A new method is being developed for purification of C2 by a rapid technique. Considerable effort in purification has also lead to major advances in the ability to prepare and purify C5a, the major chemotactic fragment of complement and the fragment that is responsible for much of the anaphylatoxic activity of complement in serum. In collaboration with Drs. Kim Yancey and Tom Lawley, we have begun to examine the function of C5a on injection into man and to characterize the products of this reaction. The cellular response evoked by injection of C5a into man has been defined as have been the kinetics of edema formation, etc. The role of C5a in dermatologic disease is now under active investigation. Also within the past year has come the realization that monocytes previously thought not to possess a particular complement receptor, the C3d receptor, do express this receptor, particularly when the cells have been allowed to adhere to surfaces. This receptor appears to have biologic function and ongoing experiments suggest that it may augment the phagocytosis of IgG coated

particles. Other studies of receptor function are underway. Dr. Brown and his colleagues have demonstrated that both circulating neutrophils and monocytes have fibronectin receptors in confirmation of earlier studies. They have demonstrated that fibronectin interacting with the receptor on monocytes has an important effect in that it allows other receptors, such as the C3b receptor, to not only bind C3 coated particles but to phagocytose them. Thus the fibronectin receptor when occupied gives instructions to the cell on how to process materials via other independent receptors. In this way, fibronectin acts as an opsonin without actually interacting with the particle to be phagocytosed, a new concept in opsonization. Studies by Dr. Fries have further defined the Fc receptor present on monocytes in patients with various diseases. These studies have now demonstrated that women have a larger number of Fc receptors on their circulating monocytes than do men and that the number in both sexes increases markedly in patients with Systemic Lupus Erythematosus. The average number of receptors and average association constant which decreases slightly in these patients can be related to disease activity. Also, it has been shown that patients with Chronic Granulomatous Disease have about half the number of C3b receptors on their surface as do normal cells, although patients with many other chronic infections have an increased number of receptors. Patients with Job's syndrome characterized by high levels of IgE and frequent infections have decreased C3b receptors, in this case, both on their polymorphonuclear neutrophils and on their circulating erythrocytes.

The characterization of the bacterial response to the late components of complement has continued. In general, the acquisition by bacteria of serum resistance, that is resistance to the lytic action of complement, is associated with a marked increase in virulence and many organisms which disseminate to various parts of the body are characterized by their serum resistant surface. Dr. Joiner and his group have further characterized this process during the past year. Earlier studies had shown that on certain model serum resistant organisms, complement component complexes build up on the surface of the organism becoming increasingly hydrophobic as they do so. These hydrophobic complexes insert into the lipid bilayer of the bacterial membrane disrupting the cytoplasmic membrane and killing the bacterium. In the resistant organism, instead of inserting into lipid domains, the membrane attack complex is shed from the organism surface. In recent studies with gonococci, it has been shown that, although the organism does not shed C5-9 from its surface to prevent insertion into lipid domains, it appears to allow association of the 5-9 complex with cell wall elements that do not allow appropriate lipid insertion and lysis. Under these circumstances the organism with the membrane attack complex on its surface is stable and capable of growth. The specific surface compounds associated with prevention of membrane attack by the 5-9 complex are under study at present. It has also been shown that in certain encapsulated organisms the presence of capsule prevents the C5-9 from being released from the organism's surface, although it assembles and fails to insert in these resistant *E. coli* in the same way that it fails to insert into the resistant model *Salmonella* studied earlier. The role of antibody in activation of the attack complex has also been examined in ongoing studies. In these model systems, it has been shown that antibody appears to focus the membrane attack complex in such a way that the membrane attack complex causes lysis when it binds to the organism in the presence of antibody and binds to the organism without a lytic event in the absence of antibody. The biochemical basis for this phenomena is under study. Experiments have also examined the molecular basis of PNH and particularly the mechanism of aplastic anemia. It has been shown that PNH patients have a marked decrease in the number of early erythroid

precursors with an additional marked increase in the turnover of these early precursors. This turnover may lead to depletion of the precursor pool, explaining the propensity for aplasia in this patient group.

The Mucosal Immunity Section

The Mucosal Immunity Section continues its studies of the mechanism of mucosal immunity and the role of the immunologic apparatus in causing Crohn's disease and other related illnesses. Immunoregulatory defects in patients with Crohn's disease have been further characterized. These patients have excessive suppression of immune responses by their cells when studied in vitro. It was possible to show that this excessive suppressor effect of peripheral blood lymphocytes in Crohn's disease is due to the acitivity of a newly defined population of cells having a specific phenotype which can be recognized by monoclonal antibodies. This should facilitate the further definition of the cause of this disease. It is interesting that cells isolated from the lamina propria of patients with this disease have a small percentage of these cells with excessive suppressor activity. Further studies of cells isolated from patients with this disease are ongoing. The conditions which govern the secretion of immunoglobulin by cells within the gut have also been studied. It is well known that these cells secrete immunoglobulin A, an important component of the host defense mechanism. Earlier studies had suggested that this secretion was dependent on a cell-controlled switch mechanism which turned on cells to secrete this immunoglobulin. The details of the switch mechanism and factors which lead to not only IgA membrane production but also IgA synthesis and secretion have been defined during the year. The disease Primary Biliary Cirrhosis has also been examined in some detail. An immunoregulatory defect has been defined in this important inflammatory disease of the liver. It has been shown that patients with this disorder have a defect in function of their natural killer cells which are responsible for recognizing and killing certain abnormal cells in experimental studies and are thought to be important in tumor cell surveillance in vivo. Definition of this defect during the past year has lead to the suggestion that, in fact, the numbers of cells are normal but their function is not. This defect is being further characterized. As part of these studies, considerable attention has been paid to a phenomena called the autologous mixed lymphocyte reaction. In this reaction, cultured B-cells and T-cells obtained from the same patient are capable of responding to each other with T-cell proliferation. This phenomena does not occur normally in Primary Biliary Cirrhosis. The studies suggest that, in fact, the autologous MLR does not lead to the generation of cells capable of self killing in normal individuals and suggest that it is part of a complex immunoregulatory mechanism. As part of these studies, long term autoreactive cell lines which contain cells capable of killing autologous target cells have been developed. The inability to demonstrate such cells in the past is probably due to the fact that the relevant reacting cell was present at too low a concentration in the cultured cell line for measurement. The ability to form such cell lines with this type of reactivity in vitro will allow for simplified study of this phenomenon.

The Allergy Program

The Allergy Program has had another excellent year. Specific projects are detailed in the relevant section of the detailed reports. Studies have examined

in vitro phenomena, such as mechanisms of histamine release and the mechanisms of mucus secretion by cells in culture. They have also explored animal models of in vivo hypersensitivity. In addition, a considerable number of studies have examined patients with various kinds of allergic disease.

Dr. Kaliner's group has spent considerable time analyzing, in detail, the late phase reaction following allergenic challenge. This is a reaction which occurs some hours after the immediate hypersensitivity reaction due to edema formation secondary to mediator release. Late phase reactions were shown to require the participation of neutrophils for full expression and could be prevented by treatment with H1 and H2 antihistamine combinations. This lead to a characterization and quantitation of the number of histamine receptors on various cell populations including neutrophils, lymphocytes and purified lung membranes. This in turn led to the need for developing histamine assays of greater sensitivity and specificity. It was shown that histamine levels were constantly elevated in a group of patients being followed with Systemic Mastocytosis. Moreover, an assay for nasal blood flow was developed using modern laser technology. It was shown that Allergic Rhinitis patients have the same rate of blood flow as do normals. This blood flow through the nose was reduced by alpha adrenergic but not affected by cholinergic stimulation. This kind of technology, looking at local blood flow, will allow very interesting analysis of the effect of various mediators on flow and capillary permeability. In studies of mucus secretion, in vivo techniques have been employed to determine the parameters which affect goblet cell formation of mucus. Mucus formation with bronchial plugging is one of the prime causes of the pathology of asthma and there are few careful studies to show what determines the synthesis and degradation of mucus. In this section, models for measurement of mucus production by cultured human bronchial and nasal mucosa were developed and the role of various mediators is being examined in a systematic, quantitative fashion. Early studies showed that macrophages produce a low molecular weight substance after phagocytosis which causes marked mucus secretion. The substance has been termed macrophage mucus secretagogue. This low molecular weight substance may be important in promoting mucus secretion at the area of inflammation. In studies by the group, the various substances which control mucus secretion are being systematically explored. Prostaglandins have been shown to influence the secretion of mucus and more recently, the leucotriene series of compounds has been shown to have marked mucus secretion stimulating ability.

Dr. Metcalfe has continued his studies of mast cell degranulation and the ingestion of the mast cell granules by connective tissue fibroblasts with their subsequent degradation. The kinetics and details of this degradation process have been examined. It has been shown that mast cell products can alter fibroblasts responses such as enzyme secretion and proteoglycan synthesis. In vitro studies also demonstrated that mast cell granules by virtue of their heparin content can inhibit lymphocyte function. The role of the proteoglycan in mast cell function is being explored in detailed studies with the hope of defining the role of this remarkably interesting series of compounds in allergic and immunologic responses.

Dr. Metcalfe's group has become one of the leading groups in the world in analysis of food hypersensitivity in man. Patients with positive skin tests or positive histories of food allergy have been studied following IgE levels, plasma and urinary histamine, and clinical signs and symptoms have been correlated. Those situations in which food challenges are associated with positive skin tests

and/or positive serum tests have been defined. As part of this, a new and highly sensitive method for IgE specific antibody has been developed allowing for greater definition of the number of allergens and the response to allergenic stimulation. Detailed studies are progressing on the difference between mast cells as found in tissues and mast cells found in other sites such as the intestinal tract, which appear to be a different population of cells than those in other areas, and circulating basophils. These differences are being examined and now it is possible to biochemically define differences between these three cell types. Studies have also been carried out on patients with Systemic Mastocytosis, Urticaria Pigmentosa, and Recurrent Idiopathic Anaphylaxis. Urine histamine levels were significantly elevated in patients with each of these diseases, however, plasma histamine was only increased in Systemic Mastocytosis. Methods of therapy have been and are being defined in these different mast cell hypersecretory states. Clinical studies suggest that bone scans in ten cases of Systemic Mastocytosis were correlated to plasma histamine levels. Four bone scan patterns were noticed: normal, focal, multifocal, and diffuse. Plasma histamine levels were progressively greater as the scan pattern progressed from normal to diffuse. Thus, bone scintigraphy was useful in both diagnosis and assessing the severity of Systemic Mastocytosis.

The three elements of the Laboratory of Clinical Investigation thus combine to offer insights into the host defense reaction and the host defense mechanism for destroying foreign invaders. Presumably, allergy represents a disorder of this system in which foreign substances to which the individual is exposed provoke a reaction in the patient which leads to clinical symptoms. It is presumed that this response has been developed to fight microorganisms, or parasites. As the factors which control these pathophysiological disorders become known, it should be possible to define better methods for dealing with patients who have disorders in the host defense mechanism on one hand, and developing immunoregulatory means of controlling allergic symptomatology on the other.

HONORS AND AWARDS

Dr. Frank has completed three years as Secretary-Treasurer of the American Society for Clinical Investigation. He was asked to deliver the Solomon Papper Lecturership in Humane Scholarship at the University of Oklahoma in February 1983. He has served on the Scientific Directorate of the Leonard Wood Memorial dedicated to medical research to understand and control leprosy. Dr. Frank was invited to be a member of the Editorial Board for the journal, Proceedings of the Society for Experimental Biology and Medicine and for the book series Advances in Host Defense Mechanisms. Dr. Gallin serves as an editor of that series. Dr. Frank was also invited to be an editor of the important Clinical Immunology textbook, Immunologic Diseases by Max Sampter. He was asked to chair the only complement symposium at the International Complement Meeting in Kyoto to be held in August 1983 and was asked to chair sessions at the Complement Workshop, the Gordon Conference on Phagocytes and the Federation of Societies in Experimental Biology Meeting.

Dr. Michael Kaliner presented the David Talmadge Commemorative Lecture at the Annual Aspen Allergy Conference in July 1983. He was elected President-Elect of the Allergy and Immunology Assembly of the American Thoracic Society. He was elected Chairman of the Examination Committee of the American Board of Allergy and Immunology and placed on the MKSAP VII committee on allergy and immunology, which re-certifies physicians.

Dr. Dean Metcalfe was named to the American Academy of Allergy journal, the Journal of Allergy and Clinical Immunology and on that editorial board he joins Dr. Kaliner and Dr. Frank.

Dr. John Bennett continues as a member of the Editorial Board of the Journal of Infectious Disease and as a counselor of the Infectious Diseases Society.

Dr. John Gallin was elected a member of the American Association of Physicians. He continues to serve as an editor for many journals including Inflammation, Advances in Host Defense Mechanisms and the Journal of Clinical Investigation. He continues as an organizer of the biyearly symposium in "Infection in the Immunocompromised Host".

Dr. June Kwon-Chung was asked to lecture at the International Conference on Mycology held in Japan, July 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00043-18 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunology and Chemotherapy of Systemic Mycoses

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

John E. Bennett, M.D., Head, Clinical Mycology Section, LCI, NIAID

COOPERATING UNITS (if any)

Dr. A. Bhattacharjee and C. Glaudemans, LC, NIAMDD

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

4.4

PROFESSIONAL:

2.4

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients cured of cryptococcal meningitis did not respond to immunization with cryptococcal polysaccharide either as measured by serum antibody or presence of circulating blood lymphocytes (PBL) which spontaneously secreted anticryptococcal antibody. Response to type III pneumococcal polysaccharide was normal by both parameters, as was immunoglobulin secretion by mitogen-stimulated PBL. These patients have long-lasting specific tolerance to polysaccharide from the infecting fungus.

Rabbit IgG antibody to the capsular polysaccharide of type D Cryptococcus neoformans had affinity for both the xylose and glucuronate side chains but not the mannan core. Computer modeling of the polysaccharide structure offered an explanation for these findings.

An experimental model and mode of analysis was devised to show that the often impuned flucytosine in vitro susceptibility test correlated with in vivo protection of mice infected with Candida albicans.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00045-15 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Interaction of Antibody and Complement on Production of Immune Damage

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Michael M. Frank, M.D., Head, Clinical Immunology Section and Chief, LCI/NIAID

COOPERATING UNITS (if any)

Neal Young, M.D., IR/CHB/NHI

Jeff Moore, M.D., IR/CHB/NHI

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

5.2

PROFESSIONAL:

3.2

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

No simple method has previously been successful for preparation of C1 inhibitor. Efforts to purify the C1 esterase inhibitor have now been successful and have allowed the preparation of a fully functional molecule with twice the activity/mg of serum C1 inhibitor. Four molecules of this inhibitor inhibit one molecule of activated C1. A new purification procedure uses Zn⁺⁺ chelate chromatography, never before used to prepare this protein. The plan is to perform turnover studies in man with this material.

Immunoregulatory defects have been defined in Hereditary Angioedema. These patients have major autoimmune manifestations on serologic testing. They have few signs of severe clinical autoimmunity, although they have frequent mild signs, such as Sicca Syndrome, granulomatous bowel disorders, etc. The basis for this immunoregulatory defect is being investigated at present.

Studies of PNH continue. It has been shown for the first time that these patients have an abnormality of the primitive erythroid stem cells (BFUE), probably as a result of stem cell complement sensitivity and resulting lysis of these cells. Unlike almost all other hemolytic states, which affect cells later in development (CFU-E), these early cells are turning over rapidly in PNH and are presumably susceptible to destruction. This may explain the propensity to an aplastic peripheral blood picture in this patient group.

We have demonstrated that peripheral blood monocytes express a receptor for the complement fragment iC3b when they differentiate following attachment to glass or plastic surfaces. Efforts are underway to define the functional significance of this receptor in terms of their contribution to the host defense process. Particular attention at present is paid to their function in promoting phagocytosis.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00047-14 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies of Patients with Known or Suspected Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Eric A. Ottesen, Head, Clinical Parasitology Section, LCI/LPD, NIAID

COOPERATING UNITS (if any)

See Next Page

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Parasitology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of these studies is to increase understanding of the diagnostic, therapeutic and pathogenic aspects of parasitic infections.

Work with helminth infections has identified species specific antigens as well as particular types of antibody responses (IgE and individual IgG subclasses) that show enhanced specificity in the diagnosis of filariasis, schistosomiasis and strongyloidiasis. Sensitive diagnostic methods for detecting parasite antigens circulating in the blood (filariasis, schistosomiasis) or found in the stool (giardiasis) have also been developed.

Therapeutic studies of patients with cutaneous leishmaniasis indicate that local heat treatment for isolated lesions is effective in selected cases.

Long-term studies of the efficacy of diethylcarbamazine (DEC) used prophylactically (loiasis) or chronically (bancroftian filariasis) either with or without other drugs are underway in Africa and India. The violent side effects of DEC treatment in patients with onchocerciasis appear to be initiated by complement activation that leads to destruction of microfilariae followed by widespread immediate hypersensitivity reactions.

The pathology of parasitic infections results from both the patient's immune responses to the parasite and the pathogenic potential of the parasite itself. For helminth infections (filariasis, schistosomiasis, strongyloidiasis) cellular and humoral (especially IgE) immune responses and their specific regulatory mechanisms have been characterized. Work on the pathogenicity of protozoal infections (giardiasis, leishmaniasis) has focused more on strain differences among the parasites and their roles in determining both the character of the pathology induced in patients and the clinical response of these patients to treatment.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00048-13 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Pathophysiology of Autoimmune Hemolytic Anemia

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Michael M. Frank, M.D., Head, Clinical Immunology Section and Chief, LCI/NIAID

COOPERATING UNITS (if any)

Thomas Lawley, M.D., DB/NCI

P. Plotz, M.D., NIAMDDK

W. Mullens, M.D., NIAMDDK

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 - (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies continue on the role of the reticuloendothelial system in the clearance of immune complexes and in the production of autoimmune disease. Particular emphasis has been placed on Fc receptors, their enumeration, and mechanism of action. Last year we reported the development of methods designed to determine average number of Fc receptors on peripheral blood monocytes and their average association constant. A large group of patients with autoimmune hemolytic anemia were shown to have a marked increase in their cell membrane Fc receptor number with a normal association constant of their receptor protein. On steroid therapy, the receptor number decreased toward normal. The role of glucocorticoids in receptor number and clearance rates was studied in normal volunteers. It was shown that the number of Fc receptors/cell on peripheral blood monocytes was down regulated by 4 to 5 days of 1 mg/kg Prednisone. Studies were conducted on patients with active systemic lupus erythematosus. These patients were shown to have a marked increase in peripheral blood monocyte Fc receptor number with a modest decrease in receptor association constant, both of which correlate with disease activity.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

701 AI 00057-10 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic Studies on Pathogenic Fungi

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

K.J. Kwon-Chung, Research Microbiologist, LCI/NIAID

COOPERATING UNITS (if any)

A. Pert (NIMH)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Mycology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Topics of current studies include: 1) determination of glycine assimilation and canavanine sensitivity in the two varieties of Cryptococcus neoformans, 2) finding the reasons why most of C. neoformans var gattii isolated are avirulent for mice, 3) finding an effect of catecholamine concentrations of animal brain for the growth of C. neoformans and 4) taxonomic studies on the cerebral mycotic agents belonging to the genus Cladosporium. Biochemical study showed that 100% of C. neoformans var. gattii (B&C serotypes) utilize glycine as the sole source of carbon as well as nitrogen, while 19 and 11% of A and D serotypes, respectively, could utilize the amino acid. The glycine utilizing isolates were found to metabolize glycine via serine and pyruvate. One hundred per cent of C. neoformans var. gattii were also found to be resistant to L-canavanine up to 13 ml while 100% of serotype D and 30% of serotype A of C. neoformans var. neoformans were sensitive to the drug. Protein and DNA synthesis were not affected by the drug in the resistant isolates while they were inhibited in the sensitive isolate. Rate of the drug uptake was the same in the two groups. Unlike C. neoformans var. neoformans, C. neoformans var. gattii spontaneously produced temperature sensitive, phenoloxidase negative subpopulations in a high proportion. These populations were avirulent for mice. The rats with catecholamines in brain permitted slower growth of C. neoformans than the control rats. The long lost Cladosporium bantianum was isolated from a cat lesion and was found to be significantly different from C. trichoides. A new variety, Cladosporium trichoides var. chlamydosporum isolated from a human brain abscess was described.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00058-09 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Pathogenesis and Chemotherapy of Herpesvirus Infections in Man

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

S.E. Straus Senior Investigator, LCI, NIAID

COOPERATING UNITS (if any)

J. Hay (USUHS), W. Ruyechan (USUHS), R. Whitley (Birmingham, Alabama).
H. Masur (CC, NIH), G. Quinnan (BOB/FDA), G. Tosato, M. Blaese (MET/NCI)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.5

PROFESSIONAL:

1.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The pathogenesis, molecular biology, immunology, natural history, and therapy of human herpes virus infections are being investigated. Immunocompetent and immunodeficient patients with a wide range of herpesvirus infections have been identified, followed clinically, diagnosed by virus isolation, and studied immunologically. During the past year we have made major advances in the treatment and suppression of herpes simplex infections using acyclovir.

Our major focus on the study of the molecular biology and latency of varicella zoster virus (VZV) DNA has continued. We have completed restriction endonuclease analyses and molecular epidemiologic studies of 26 strains of VZV DNA and have completed the mapping of the VZV genome. We are now using these recombinant clones for rapid diagnosis of varicella and zoster infections and as probes of human tissues to determine the sites and nature of viral latency. In the coming year we will initiate studies of the genetic expression of VZV.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00154-08 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Events in Immediate Hypersensitivity

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Michael A. Kaliner, Head, Allergic Diseases Section, LCI, NIAID

COOPERATING UNITS (if any)

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Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7	4	3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our analysis of events in immediate hypersensitivity focuses on human and animal models of allergic responses, mechanisms of mediator action, and pharmacologic approaches to allergic diseases. Immediate hypersensitivity reactions are followed by late phase allergic responses (LPR), for which a rodent model has been developed. LPR require the participation of neutrophils for full expression and may be prevented by pretreatment with H-1 and H-2 antihistamines. In order to examine histamine responses, histamine receptors on human neutrophils, lymphocytes, and purified lung membranes have been quantified. All three tissues have a large number of relatively high affinity H-1 receptors. Very specific and sensitive assays for histamine reveal that urine histamine increases significantly after intravenous radiocontrast media and plasma histamine levels are constantly elevated in systemic mastocytosis. An assay for nasal blood flow has been developed employing laser-doppler velocimetry. Allergic rhinitis subjects have the same blood flow as normals, blood flow is reduced by alpha adrenergic agonists and not affected by cholinergic stimulation. In vivo analysis of vascular permeability may be a unique approach to the study of urticaria; therefore, measurement of vascular leakage of proteins and dextrans of several sizes has been developed. A model for depletion of mast cells involving ricin linked to myeloma IgE has been developed.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00155-08 LCI

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phagocytic Cell Function

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

John I. Gallin Head, Bacterial Diseases Section LCI, NIAID

COOPERATING UNITS (if any)

Dr. M. Friedman (Dept. Microbiol. Georgetown Med. Ctr.),
Dr. J. A. Charon (NIDR, NIAID), Dr. A. G. Palestine (NEI)

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

6.4

PROFESSIONAL:

3.9

OTHER:

2.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The modulation of neutrophil (PMN) function has been studied by assessing chemoattractant receptors, cytoskeleton function, chemotaxis, degranulation and oxidative metabolism in normal and abnormal cells. The data indicate there is an intracellular pool of receptors for the chemoattractant fmet-leu-phe with the density of specific granules that are mobilized to the plasma membrane with cell activation. These data, together with studies in neutrophil cytoplasts, support the concept that specific granules are a reservoir of receptors and show that receptor mobilization is independent from receptor affinity adaptation.

In studies of factors regulating chemotaxis, high extracellular potassium, was shown to activate PMN capping of lectin receptors and induce shape changes, similar to chemoattractants. Since potassium would be expected to be increased at inflammatory foci, where there are dead or dying cells, the data suggest potassium may exert an important modulatory role on the inflammatory response. Abnormal "turn-off" of acute inflammatory reactions was seen in chronic granulomatous disease (CGD) with persistence of PMN late in experimental inflammation. This indicates different biochemical lesions can be manifest as phenotypic disease. Other clinical studies revealed that incubation of monocytes from patients with the Hyperimmunoglobulin E-Recurrent infection (Job's) syndrome with *S. aureus* causes release of an inhibitor of PMN chemotaxis. In addition, sera and saliva from patients with Job's syndrome was shown to be deficient in antistaphylococcal IgA.

New approaches for pharmacologic manipulation of PMN function were explored. Auranofin, an oral gold compound used to treat rheumatoid arthritis, induced high affinity states of the PMN receptor for fmet-leu-phe and preferentially inhibited oxidative metabolism and lysozyme secretion. The data suggest pharmacologic control of PMN chemoattractant receptors may provide a tool for selective manipulation of functions activated by these receptors.

18-20

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00189-04 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1982

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Biochemical Studies of Human Enteral Adenovirus Infections.

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Stephen E. Straus, Senior Investigator, LCI, NIAID

COOPERATING UNITS (if any)

C. Brandt and W. Rodriguez (Children's Hospital, D.C.) H.S. Ginsberg
(Columbia Univ.), R. Yolken (Johns Hopkins), J. Esparza (Caracas, Venezuela)

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Laboratory of Clinical Investigation

SECTION

Medical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Enteral adenoviruses (EAd) comprise two related serogroups of viruses which appear to cause gastroenteritis in infants. Studies of these viruses and the diseases they produce were hampered until recently by an inability to grow them in tissue culture. Using a method we have developed for growing these viruses, numerous isolates from around the world have been studied. EAd DNA fragments have been cloned in plasmid vectors, and DNA restriction maps of serogroup F and G strains have been completed. Analyses of the genomic structures of these viruses demonstrated partial sequence homologies to conventional adenoviruses. Recombinant clones derived from areas showing little or no homology to non-EAds have been used to develop a highly sensitive and specific dot-blot assay for EAds in clinical specimens. Our current efforts include further molecular epidemiologic studies, a search for EAds and Ads in AIDS patient specimens, characterization of EAd and Ad binding to specific tissues, and mapping of early viral MRNAs.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00192-05 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Immediate Hypersensitivity

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Dean D. Metcalfe, M.D., Senior Clinical Investigator, LCI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mediator-containing mast cell granules have been shown to be ingested by connective tissue fibroblasts. This phagocytic process is both time and temperature dependent, and results in an increased rate of enzyme secretion from fibroblasts. The heparin matrix of the granule is degraded within the fibroblast following ingestion. This ability of fibroblasts to remove and degrade mast cell granules represents an important mechanism by which immediate hypersensitivity reactions are terminated. It also appears that mast cell products can alter fibroblast responses such as enzyme secretion and proteoglycan synthesis.

In studies using human lymphocytes, mast cell granules were found to inhibit antigen- and lectin-induced blastogenesis. This effect persisted after histamine removal. The inhibitory factor was shown to be heparin proteoglycan. Thus, mast cell granules, by virtue of their heparin content, can inhibit lymphocyte function.

Proteoglycans from three cloned granulated lymphocyte cell lines with natural killer activity and one cultured mast cell line were characterized. The proteoglycans from each cell line were distinct, with the granulated lymphocytes containing only chondroitin 4-sulfate and the mast line producing an oversulfated chondroitin 6-sulfate. These findings suggest that glycosaminoglycan profiles are useful biochemical markers in the characterization of diverse granulated cell lines.

Cultured mast cells derived from mouse bone marrow produce an oversulfated chondroitin sulfate rather than heparin. This observation, along with the presence of characteristic surface markers and distinct responses to degranulating agents suggests that these cells are mucosal mast cells.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00249-02 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Pathogenesis, Diagnosis, and Treatment of Systemic Mast Cell Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Dean D. Metcalfe, M.D., Senior Clinical Investigator, LCI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.75	

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Plasma and urine histamine levels were determined in patients with systemic mastocytosis, urticaria pigmentosa, and recurrent idiopathic anaphylaxis.

Urine histamine levels were significantly elevated in patients with each of the three diseases when compared to histamine levels in normal subjects.

However, plasma histamines were increased only in the systemic mastocytosis patients. Such elevations were persistent over the 15 to 25 weeks of follow-up. There was a significant relationship between plasma and urine histamine when measured concurrently. Thus, increased plasma histamine appears to differentiate systemic mastocytosis from other systemic disorders of mast cells.

Scintigraphic findings in 10 cases of systemic mastocytosis were correlated to plasma histamine levels. Bone scans showed more widespread involvement than radiographs in patients with diffuse disease and detected a greater number of focal lesions. Four bone scan patterns were noted: 1) normal, 2) focal, 3) multifocal, and 4) diffuse. Plasma histamine levels were progressively greater as the scan pattern progressed from normal to diffuse. Thus, bone scintigraphy is useful in both the diagnosis and in assessing the severity of systemic mastocytosis.

The value of combination H₁ and H₂ antihistamines or cromolyn sodium in the symptomatic relief of systemic mastocytosis was assessed. Neither drug regimen significantly differed in its efficacy, although antihistamines appeared somewhat more useful in relieving pruritis. Neither drug combination altered plasma histamine levels.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00250-02 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Basic Studies on Inflammatory Diseases of the Gastrointestinal Tract

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Dean D. Metcalfe, M.D., Senior Clinical Investigator, LCI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:

2.15 2.15

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In a clinical study on immediate adverse reactions to foods in adults, a positive skin test to a given food correlated with reproduction of symptoms on double-blind food challenge. Patients whose symptoms could be reproduced tended to be atopic, have multiple positive skin tests to foods and inhalants, and to have higher IgE levels. Plasma and urine histamine levels generally remained normal during clinically positive food challenges unless the reaction was generalized. Positive reactions often involved the gastrointestinal tract, respiratory tract, and skin. Specific IgE to foods did not rise significantly after challenge.

Cultured mouse mast cells with characteristics resembling those of mucosal mast cells have been examined for their response to degranulating agents and compounds known to modulate release. These cells released histamine to immunologic stimuli, concanavalin A, ionophores, and substance P, but were refractory to several other agents including compound 48/80, gastrin, and neurotension. Release was inhibited by theophylline, but not cromolyn sodium. Experiments are underway to isolate mucosal mast cells directly from animal intestinal mucosa using a combination of enzymatic dispersion, elutriation, and gradient centrifugation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00269-02 LCI

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phagocytic Cell Activation

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Bruce E. Seligmann

Staff Fellow

LCI, NIAID

COOPERATING UNITS (if any) Dr. Thomas Chused (LMI, NIAID), Dr. Harry Malech, Yale University School of Medicine, Dr. Marc Friedman, Department of Microbiol., Georgetown Medical Center

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD

TOTAL MANYEARS:

1.1

PROFESSIONAL:

1.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Modulation of phagocyte activation by the synthetic chemoattractant fmet-leu-phe has been studied using membrane potential and calcium sensitive fluorescent probes, and assays of superoxide generation and degranulation in PMN. Our data indicate modulation of fmet-Leu-phe receptor affinity may be an important mechanism by which PMN adapt and respond to a gradient of chemoattractant during the process of chemotaxis. Related studies of neutrophil heterogeneity, which we described previously, revealed heterogeneity of fmet-leu-phe binding. All cells bind fmet-leu-phe to their plasma membrane in a nondisplaceable manner, in addition to internalizing the ligand. The subpopulation of PMN which depolarizes bound more peptide, and only these cells exhibit displaceable binding of fmet-leu-phe. Nondepolarizing cells could be induced to respond and display displaceable binding by pretreatment with cytochalasin B. Thus, functional heterogeneity exists among PMN and studies are underway to further define the significance of this finding. In other studies the effect of amphotericin B was determined. At concentrations achieved *in vivo* this drug causes PMN to depolarize and secrete their granule contents, produce superoxide anion, hydrogen peroxide and to aggregate. Thus, amphotericin B is a secretagogue for PMN and this may contribute to its toxic effects *in vivo*.

The effect of histamine on PMN function has also been investigated. It was found that histamine reversibly and specifically inhibited oxidative metabolism, chemotaxis and membrane potential changes stimulated by fmet-leu-phe, but was without effect on functions stimulated by other agents such as phorbol myristate acetate. The histamine receptor which mediated these effects demonstrated affinity for both H₁ and H₂ analogues and only H₂ antagonists (cimetidine) were capable of reversing the inhibitory effects of histamine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 A1 00270-02 LCI

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tubulin Tyrosinolation in Normal and Abnormal Human Neutrophils

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Jayasree Nath, Ph.D.

Expert

LCI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Bacterial Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The biochemical modification of human neutrophil (PMN) microtubules/tubulin was studied by monitoring the specific stimulation of the post-translational incorporation of tyrosine into tubulin α -chains, by the chemoattractant fmet-leu-phe and also by the Ca^{2+} -ionophore A23187. The data indicate that both fmet-leu-phe and A23187 stimulate PMN tyrosinolation, which is abolished under anaerobic conditions and in the presence of various anti-oxidants and reducing agents. Studies in PMN from patients with the Chediak-Higashi syndrome and with chronic granulomatous disease indicated a greatly exaggerated response in the former and inhibited response in the latter. Based on studies of the oxidative state of the patients' cells and studies using oxidizing and reducing agents, it is concluded that there is a close association between PMN redox state and tubulin tyrosinolation. Results of recent studies also indicate the requirement of extracellular free Ca^{2+} and the cellular Ca^{2+} -regulatory protein calmodulin, in the modulation of tubulin tyrosinolation in PMN. The data provide new insights into the relationship of PMN redox state and free Ca^{2+} concentration in modulation of tubulin tyrosinolation and neutrophil function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00271-02 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Purification and Characterization of Serum Complement Proteins and Fragments

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Carl H. Hammer, Ph.D., Senior Investigator, LCI/NIAID

COOPERATING UNITS (if any)

Dr. Moon L. Shin, Dept. of Pathol., Univ. of MD, School of Med., Baltimore, MD

Dr. Jerry Winkelstein, Dept. of Pediatrics, Johns Hopkins School of Medicine, Baltimore, MD

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

1.45

PROFESSIONAL:

0.45

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Refinements developed during five large scale preparations of multiple complement components have allowed us to improve resolution and functional recovery of C3 (96%), C4 (57%) and C5 (65%) and to maintain C9 yield. A second stage DEAE-Sephadex column was used in which certain components (P, I, C2, B, C7, C8, and C6) obtained now as complex pools are rechromatographed under more favorable conditions. This step generated relatively pure components more amenable to purification. C1-In has been purified to homogeneity. C2 and C4 are in final stages of purification and further purification of C6 is under way. A C3 deficient strain of dogs was recently identified. To study this C3 deficiency, methods to isolate C3 from normal dogs were developed and resulted in the preparation of pure C3 determined to have a M.W. of 180,000. Further work has resulted in methods to produce and recover highly purified C5a from citrated plasma. This C5a migrates as a 15,000 dalton band on SDS-PAGE. Staining with antibody to C5 immunoblots of C5a on nitrocellulose confirm the presence of this biologically active peptide. Studies on cell-bound C4b suggest that the alpha-chain as well as the beta-chain may be important in generation of C3 convertase. Lysis of nucleated cells by complement demonstrated a cooperative action of terminal complement components in contrast to one-hit kinetics for small marker release. This divergence can be interpreted as a multi-channel requirement for killing these nucleated cells.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00272-02 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Host Defense Against Pneumococcal Bacteremia

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Eric J. Brown, M.D., Senior Investigator, LCI/NIAID

COOPERATING UNITS (if any)

Melvin Berger, M.D., Walter Reed Army Medical Center

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Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

1.75

PROFESSIONAL:

0.75

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have demonstrated that the classical complement pathway is essential to mediate antibody opsonization of pneumococci. We have compared antibody against different components of the cell surface and capsule in their ability to cause C3b binding to pneumococci and shown them to be equivalent. We have compared these C3b deposited in different loci for their ability to bind beta-1H, factor B, factor I and the erythrocyte C3b receptor. These studies have demonstrated on a molecular basis the explanation for the observations that 1) pneumococcal cell walls activate the alternative pathway of complement; 2) pneumococcal capsules do not activate the alternative pathway and 3) anticapsular antibody is more opsonic than anti-cell wall. We have also demonstrated that antibody on pneumococci is a significant acceptor site for C3b during classical pathway activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00273-02 LCI

PERIOD COVERED

Period covered
October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders)

The Role of Fibronectin in Opsonization and Phagocytosis

PRINCIPAL INVESTIGATOR (*List other professional personnel on subsequent pages.*)

(Name, title, laboratory, and institute affiliation)
Eric J. Brown, M.D., Senior Investigator, ICI/NIAID

COOPERATING UNITS (if any)

Thomas Chused, LMI, NIAID

Celso Bianco - New York Blood Center

Theodore Breitman, LTCB, NCI

Tsuneo Takahashi, American Red Cross

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LAB/BRANCH Laboratory of Clinical Investigation

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National Institute of Allergy & Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither

(a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies have been extended on the role of fibronectin in phagocytosis by monocytes and on phagocytosis by polymorphonuclear leukocytes and the HL60 myelomonocytic cell line. Although fibronectin causes phagocytosis of C3b coated erythrocytes by monocytes, it has no effect on phagocytosis by polymorphonuclear leukocytes. If PMN are first stimulated by C5a or f-met-leu-phe however, fibronectin has a dramatic effect on C3b mediated phagocytosis. HL60 cells will phagocytose C3b coated particles in the presence of fibronectin, if first exposed to DMSO, phorbolmyristic acetate, or lymphokine rich cell culture supernatant. The maximal effect of each of these agents appears after 3-5 days of co-culture with HL60.

A mechanism for studying fibronectin binding to cell surfaces has also been developed. Fluoresced beads are coated with fibronectin, and their binding to cells studied with flow microfluorocytometry. Both monocytes and PMN as purified from peripheral blood will bind fibronectin coated beads; lymphocytes will not. HL60 cells do not bind these beads but do bind them after exposure to agents which induce differentiation.

Monoclonal antibodies to the fibronectin molecule have been prepared. Of approximately 10 such monoclonals tested, two antibodies inhibit binding of Fn coated beads and also inhibit Fn mediated phagocytosis. Both monoclonals are IgG, and inhibit PMN and monocyte binding equally. The monoclonals will be used to identify and isolate the fibronectin fragment which binds to monocytes and PMN and to ascertain whether or not it is identical to the fibroblast cell binding fragment.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00275-02 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Complement Receptor and C3 Mediated Opsonization

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Theilma Gaither, Research Biologist, Clinical Immunology Section, LCI/NIAID

COOPERATING UNITS (If any)

Dr. John Gallin, LCI

Dr. Victor Nussenzwieg, Dr. Kyoko Iida, NYU Medical Center

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Clinical Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

1.55

PROFESSIONAL:

1.05

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We examined PMN and erythrocytes of patients recovering from infection for the presence of the C3b receptor, CR1, using three techniques that together measure both antigenic and functional activity. We found that CR expression was markedly reduced on PMN of patients with chronic granulomatous disease and on patients with hyper-IgE or Jobs syndrome. In contrast, other infected patients studied as controls expressed significantly more PMN CR₁ than did normal healthy controls. Interestingly erythrocytes of CGD and infected control patients expressed normal levels of CR₁ whereas patients with Job's syndrome were abnormally low. The C3b receptor¹ is thought to be very important in allowing for binding and phagocytosis of opsonized bacteria. These experiments raise the possibility that these patients, with frequent infections, have an important defect in phagocytosis. The significance of these differences in CR₁ expression with regard to receptor-mediated cell function is now under study.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00276-02 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Membrane Attack Complex of Human Complement

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Keith A. Joiner, Senior Investigator, LCI,NIAID

COOPERATING UNITS (if any)

Dr. Moon Shin, University of Maryland

Dr. Barry Silverman, University of Maryland

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Clinical Immunology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL MANYEARS: PROFESSIONAL: OTHER:

1.5 1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The membrane attack complex of complement C5b-9 is responsible for complement mediated tissue damage. The formed complex can be specifically identified by neoantigens which are not present on isolated components within the complex. We had previously prepared goat polyclonal antiserum to C5b-9 neoantigens. The antiserum was absorbed extensively against pooled normal human serum which had been coupled to a solid phase matrix. The absorption procedure removes antibodies reacting with native individual constituents (C5, C6, C7, C8 and C9) making up the C5b-9 complex, but does not remove antibodies to neo-antigenic determinants. To characterize the antiserum, an ELISA was set up to detect C5b-9 neoantigens. Serum containing I-125C9 was treated with zymosan to activate complement, then subjected to sucrose density gradient ultracentrifugation. Gradient fractions were used to coat ELISA plates, and detection of neoantigen in zymosan-activated but not control serum was accomplished. Anti-neoantigen antisera were also used in immunofluorescence studies to localize C5b-9 on complement-lysed erythrocytes and tissues from patients with systemic lupus erythematosus. Attempts to prepare a monoclonal antibody to C5b-9 neoantigens have not yet been successful. In the second phase of the project, hydrophilic and hydrophobic fragments of C9 were produced by -thrombin cleavage, then separated and purified by chromatography on hydroxylapatite in 0.1% SDS. Three monoclonals raised against C9 were shown to react specifically with the hydrophobic but not the hydrophilic fragment, as assessed by Western blot. Work in progress is directed toward generating a monoclonal to the hydrophilic fragment of C9.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00277-02 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.)

Mechanism of Serum Resistance in Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Keith A Joiner, M.D., Senior Investigator, LCI, NIAID

COOPERATING UNITS (if any)

Robert Goldman and Loretta Leive, LBP, NIADDKD; John Swanson, RML, LMSF, NIAID; Robert Dourmashkin, Mount Sinai School of Medicine, N.Y., N.Y.

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National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	0.7	1.8

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies have continued on the mechanism by which specific pathogenic bacteria evade killing by the serum complement system. Studies comparing serum resistant and serum sensitive strains of Neisseria gonorrhoeae showed equivalent binding of the complement membrane attack complex (C5b-9) to the surface of these organisms. Presensitization of resistant GC with bactericidal rabbit IgG resulted in killing by serum, but killing could not be explained simply by increasing terminal complement component deposition of the bacterial surface. Sucrose density gradient ultracentrifugation of detergent extracted C5b-9 complexes indicated that the complex on sensitive organism sedimented as a major sharp 33s peak whereas the complex from the resistant strains contained broad peaks of 35s and 43s. C5b-9 co-precipitated with outer membrane constituents of sensitive and resistant GC in double diffusion and quantitative immunoprecipitation reactions. We concluded that the C5b-9 complex is in a different molecular configuration on the surface of the resistant organism than on the surface of the sensitive isolate or the resistant strain rendered sensitive with antibody. In studies with *E. coli* 0111B4 we found that bactericidal antibody is required for killing in serum, but that antibody increases primarily the bactericidal efficiency rather than the number of C5b-9 deposited on the bacterial membrane. Antibody must be present at or before C5 convertase formation to cause killing. This conclusion was based on the finding that presensitized bacteria were killed in dose-related fashion as serum concentration was raised, compared with minimal killing of non-presensitized organisms at all serum concentrations. However, binding of C3 and C9 on presensitized bacteria was not significantly different from binding on non-presensitized organisms. Antibody dependent killing was mediated via the alternative pathway by IgG and F(ab')₂ but not F(ab').

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00278-02 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Late Components of the Complement Cascade

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Carl H. Hammer, Ph.D., Senior Investigator, LCI/NIAID

COOPERATING UNITS (if any)

Dr. Moon Shin and Dr. Gertrude Hänsch
Department of Pathology
University of Maryland

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NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Complement lysis of erythrocytes (E) is mediated by C5-C9, inserting into lipid bilayers of cell membranes. We reported another mode of C5 activation which involves acidification to pH 6.4 of a mixture of C5 and C6. In the presence of C7, C8 and C9, the mixture, designated C(56)^d generates a lytic activity for chicken E. We earlier performed a comparative study of the biological behavior of C(56)^d and C5b,6 and now have extended these studies on the functional and physico-chemical properties of C(56)^d. The acid generation of C(56)^d is dose dependent on C5 and C6 and is as efficient as classical or alternative pathway activation. While C(56)^d decays in the presence of C7, the acid activated complex, is also labile at 37°C in the absence of C7. Upon ultracentrifugation, acid activated C5 and C6 sediment consistent with the formation of a bimolecular 11S complex that coincides with the appearance of lytic activity. Analysis of the chain structure of C5 obtained from C(56)^d prepared from purified components or from Egp ghosts demonstrates a C6 dependent, C5 chain cleavage. Studies on the interaction of acid-activated terminal C5b,6^d-9 with paroxysmal nocturnal hemoglobinuria (PNH)-E and NHE indicates that C5b, 6^d appears to be a principal lytic factor in acidified human serum.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00279-02 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Mucous Glycoproteins

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Michael A. Kaliner, M.D., Head, Allergic Diseases Section, LCI, NIAID

COOPERATING UNITS (if any)

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Laboratory of Clinical Investigation

SECTION

Allergic Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

1.3

1.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mucus secretion is a normal function of respiratory mucous membranes. Models for measurement of mucus production by cultured human bronchial and nasal mucosae have been developed in order to examine the controls of mucus secretion. In addition to neurohormones and mediators of allergy, airways react to a product generated by pulmonary macrophages with increased mucus release. Macrophages produce a low molecular weight substance (known as MMS or macrophage-derived mucus secretagogue) after phagocytosis which is a potent secretagogue. MMS is newly synthesized by an oligomycin-sensitive pathway, filters with an apparent molecular weight of 2000 daltons, is not an arachidonic acid derivative, and has a pI of 5.15. MMS release is stimulated by activated zymosan but not by non-activated zymosan, protein A-containing staph but not by non-protein A staph, and concanavalin A, but not by latex. MMS may play a role in mucorrhea associated with macrophage activation. Nasal turbinates secrete a family of acidic glycoproteins which range in size from $0.2-20 \times 10^6$ daltons. These glycoproteins are homogeneous in charge and isoelectric focusing (pI-2.6) and consist of 80% carbohydrate and 20% protein. Cholinergic, alpha adrenergic and immunologic stimulation increase nasal turbinate mucous glycoprotein release.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI AI 00354-01 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Immunoregulatory Defects Present in Inflammatory Bowel Disease

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Stephen P. James, M.D., Senior Investigator, Mucosal Immunity Section, LCI/NIAID

COOPERATING UNITS (if any)

Claudio Fiocchi, M.D., Cleveland Clinic Foundation, Cleveland, Ohio

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Laboratory of Clinical Investigation

SECTION

Mucosal Immunity Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD. 20205TOTAL MANYEARS: PROFESSIONAL: OTHER:
2.0 1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The immunoregulatory function of lymphocytes from patients with Crohn's disease was studied. Previous work indicated that peripheral blood lymphocytes from many patients with mild or inactive disease have excessive suppression of pokeweed mitogen stimulated immunoglobulin synthesis *in vitro*. Current studies showed that the proportion of peripheral blood lymphocyte subclasses, as defined by monoclonal antibodies Leu 2 (and OKT 8), and Leu 4 were normal, and the Leu 3/Leu 2 ratios were normal. However, the percentage of a subpopulation of lymphocytes defined by reactivity with both Leu 2 and HNK-1 (Leu 7) was found to be increased; elimination of cells reactive with OKT 8 or HNK-1 removed the excessive suppressor activity. Furthermore, the percentage of Leu2+, HNK-1+ lymphocytes correlated significantly with the suppressor function in pokeweed mitogen stimulated cultures. Thus, the excessive suppressor activity of peripheral blood lymphocytes in Crohn's disease is due to the activity of a newly defined subpopulation of cells having the Leu 2+, HNK-1+ phenotype.

To further define the role of immunoregulatory cells in Crohn's disease, we have studied lymphocytes isolated from the lamina propria of gut specimens obtained from Crohn's patients undergoing surgical resection. We have found that although the Leu 3/Leu 2 ratios are diminished compared to peripheral blood, lymphocytes from the lamina propria do not manifest excessive suppressor function, but, in fact, augment immunoglobulin synthesis. Only a small percentage of lamina propria lymphocytes from Crohn's patients have the Leu 2+, HNK-1+ phenotype which was found to be increased in peripheral blood of patients with Crohn's disease, and which correlates with excessive suppressor function.

Studies are in progress to investigate the mechanism of activation and function of subpopulations of lymphocytes isolated from the lamina propria. Further understanding of the immunoregulatory function of lymphocytes in the lamina propria of patients with inflammatory bowel disease may be important in understanding the inflammatory lesions in the bowel of Crohn's patients.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZOI AI 00 355-01 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Immunoregulatory Defects Present in Primary Biliary Cirrhosis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Stephen P. James, Senior Investigator, Mucosal Immunity Section, LCI, NIAID

COOPERATING UNITS (if any)

Anthony Jones, Liver Unit, NIAMDDK, NIH

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Mucosal Immunity Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD. 20205

TOTAL MANYEARS:

.4

PROFESSIONAL:

.4

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Primary biliary cirrhosis (PBC) is a disease of unknown etiology characterized by inflammation and necrosis of intrahepatic bile ducts, and associated features suggesting the occurrence of systemic autoimmunity. Several aspects of lymphocyte function have been investigated in patients with PBC. Previous work demonstrated that patients with PBC have diminished suppression of pokeweed mitogen stimulated immunoglobulin synthesis in vitro, and a diminished proliferative response in the autologous MLR, which in normal individuals may represent an activation pathway for suppressor cells.

In current studies, patients with PBC were found to have diminished T cell mediated suppression of immunoglobulin synthesis by Epstein Barr virus stimulated autologous B cells. In addition, patients with PBC had increased numbers of B cells in peripheral blood which spontaneously secrete immunoglobulin. These findings demonstrate that patients with PBC have an abnormality of immunoregulatory function which may involve both T and B cells.

In other studies, the diminished natural killer (NK) cell function of patients with PBC was studied. The number of effector cells, as determined by reactivity with the monoclonal antibody HNK-1, and the subpopulation of NK cells lacking T cell markers, was found to be normal. No evidence for suppression of NK activity by lymphocytes or serum factors could be found in PBC. Interferon and interleukin-2 were found to only partially reconstitute the NK defect.

Abnormal function of immunoregulatory circuits and cytotoxic cells may contribute to the pathogenesis of autoimmunity in diseases such as PBC. Further investigations will concentrate on details of the cellular mechanisms which may be the basis for these defects in PBC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00356-01 LCI
PERIOD COVERED <u>October 1, 1982 to September 30, 1983</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Studies of the Regulation of IgA Immunoglobulin Synthesis</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <u>Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI/NIAID</u>		
COOPERATING UNITS (if any) None		
LAB/BRANCH <u>Laboratory of Clinical Investigation</u>		
SECTION <u>Mucosal Immunity Section</u>		
INSTITUTE AND LOCATION <u>National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205</u>		
TOTAL MANYEARS: <u>1.0</u>	PROFESSIONAL: <u>.7</u>	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>In this project we are exploring the cellular mechanisms involved in the differentiation of IgA B cells present in mucosal lymphoid nodules (Peyer's patches, PP). In the first phase of this project we demonstrated that surface IgM (sIgM)-bearing B cells stimulated by the mitogen lipopolysaccharide (LPS) showed a high degree of differentiation into surface IgA(sIgA)-bearing B cells provided they were cultured in the presence of cloned T cells obtained from PP. In contrast, sIgM-bearing B cells stimulated by LPS in the absence of T cells or in the presence of cloned T cells obtained from spleen did not develop in sIgA-bearing B cells. PP-derived cloned T cells had thus brought about a class-specific isotype switch, and could be designated IgA-specific switch T cells.</p> <p>Since the effect of the switch T cells obtained from PP was to induce PP B cells to become cells bearing surface IgA but not cells producing IgA for secretion, it was possible that additional T cell influence was necessary for terminal differentiation into IgA plasma cells. Accordingly, in the second phase of this project (accomplished during the past year) we conducted studies in which B cells were cultured sequentially, first with LPS and cloned switch T cells, and then with fresh uncloned T cells or T cell factors derived from the latter. In these studies we found that cells exposed in the first culture to PP cloned T cells (but not spleen cloned T cells) were induced to differentiate into IgA plasma cells in the second culture if the latter contained soluble factors derived from fresh T cells stimulated with Con A or T cells themselves stimulated with staph protein A. In parallel experiments we found that cells exposed in the first culture to spleen cloned T cells were induced to differentiate into IgG plasma cells in the second culture if the latter contained the same kinds of T cell influences as before. These studies show that IgA B cell differentiation is a two-step process in which B cells are first induced to express surface IgA under the influence of switch T cells and they are then induced to differentiate into IgA plasma cells under the influence of helper T cells.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI AI 00357-01 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Autologous Mixed Lymphocyte Reaction

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Warren Strober, M.D., Chief, Mucosal Immunity Section, LCI/NIAID

COOPERATING UNITS (if any)

Gordon Yenokida, M.D., Staff Associate, Metabolism Branch, NCI, NIH

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Mucosal Immunity Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

1.3

PROFESSIONAL:

1.3

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project concerns the study of the autologous mixed lymphocyte reaction (AMLR), the proliferative response of T cells induced by exposure to autologous B cells or macrophages. In previous studies we have shown that 1) the AMLR is reduced in primary biliary cirrhosis (PBC), an autoimmune disease characterized by chronic hepatic inflammation and biliary obstruction; 2) the AMLR results mainly in the proliferation of T cells which can suppress pokeweed mitogen-driven Ig synthesis *in vitro*; 3) the AMLR is augmented tenfold if the stimulating autologous cells (B cells) are pre-activated with EB virus or non-specific mitogens. These and other data suggest that the immunoregulatory abnormality found in PBC (in other autoimmune diseases) is due to the failure to generate suppressor T cells via the AMLR.

To define further the T cells which react in the autologous MLR we have now established long term autoreactive T cell lines and have begun to define the function properties of cells in such lines. These autoreactive T cell lines were generated by repeated stimulation of T cells with autologous non-T cells (B cells and macrophages), in the presence of IL-2. The cells populations obtained respond to autologous cells but not to allogeneic cells; in addition, they do not respond to various mitogens or antigens. In initial studies we have focused on the cytotoxic capability of the autoreactive T cell lines. We have found that the Leu2-positive subpopulation (comprising 80% of the cell line) contained NK-like cytotoxicity although the cells in this subpopulation lacked markers usually associated with NK cells. In addition, we have found the the Leu 2-positive subpopulation (comprising 20% of the cell line) contained cells capable of killing autologous B cells (both activated and unactivated B cells). These studies indicate that the AMLR, contrary to previous findings, does result in the generation of cells capable of self-killing. Furthermore, they show that autoreactive lines contain cells capable of mediating NK cytotoxicity, but this is probably due to direct stimulation of NK cells by IL-2 present in the medium.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00358-01 LCI

PERIOD COVERED

October 1, 1983 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunopathogenesis of Chlamydia trachomatis Infections

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Thomas C. Quinn, Senior Investigator, LCI, NIAID

COOPERATING UNITS (if any)

Johns Hopkins University School of Medicine

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIAID, NIH Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Chlamydia trachomatis is a common sexually transmitted pathogen which causes trachoma, conjunctivitis, pneumonia of the newborn and genital infections. We have recently demonstrated that C. trachomatis may cause proctitis in homosexual men, and our recent screening studies have demonstrated positive rectal cultures for C. trachomatis in 24 (8.3%) of 288 randomly selected homosexual men and from 33 (21%) of 155 heterosexual women. Concomitant cervical infection was demonstrated in 60% of the women with rectal infections. 93% of the infected men and 72% of the infected women had signs or symptoms of proctitis. Five isolates were LGV strains of C. trachomatis and were indistinguishable clinically and histopathologically from other forms of granulomatous colitis. Non-LGV strains were associated with mild inflammatory proctitis. All chlamydial infections were associated with the rise in antibody titer with geometric mean titers of 1:1,512 and 1:282 in LGV and non-LGV infections. In summary, these studies have demonstrated that C. trachomatis may induce rectal infection which may be associated with a wide range of clinical manifestations ranging from nonspecific proctitis to severe granulomatous colitis.

In order to facilitate diagnosis and to assess for the presence of Chlamydia in pathologic tissue from AIDS and from other forms of inflammatory bowel disease, we developed an assay utilizing monoclonal antibody directed to C. trachomatis antigen which appears capable of detecting elementary bodies on cervical, urethral, and rectal swabs, and in pathologic material. Sensitivity and specificity of this assay is 97% and 98% respectively. Further studies are in progress to detect chlamydial antigen in pathologic material from AIDS patients and patients with genital and rectal infections by immunofluorescence. Studies on immunopathogenesis of chlamydial infection will be initially carried out in our recently developed experimental non-human primate models of C. trachomatis salpingitis and proctitis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00359-01 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of ELISA Assays for Intestinal Protozoans

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Thomas C. Quinn, Senior Investigator, LCI/NIAID

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TOTAL MANYEARS:

0.30

PROFESSIONAL:

0.30

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Intestinal infections with protozoan parasites such as Entamoeba histolytica, Giardia lamblia, Cryptosporidia, and Isospora belli are frequently found in homosexual men within the United States, in individuals living in tropical and subtropical areas, and in travelers returning from highly endemic areas. Diagnosis of these organisms is often difficult and requires sequential stool examination by experienced personnel. As many as 50% of infected patients have no parasites demonstrable by a single stool examination, and require additional examinations for diagnosis. Enzyme-linked immunosorbent assays (ELISA) for the detection of pathogenic antigen in stool specimens offer the advantages of being rapid and easy to perform on large numbers of specimens, standardized without difficulty and adjunctive in epidemiologic studies of these infections. We have recently developed indirect double antibody ELISA systems which detect G. lamblia or E. histolytica in single stool specimens. Studies are presently under way for the detection of Cryptosporidia and Isospora in stool specimens by ELISA. For Giardia, stool specimens were positive by ELISA in 36 of 39 (92%) of patients with giardiasis; negative specimens came from patients with low number of parasites in the stool. In ten patients followed prospectively, stools became negative by ELISA after successful treatment. Stool specimens were positive by ELISA in 3 of 128 (2%) of patients without demonstrable Giardia in their stool, but who were at high risk for giardiasis due to IgA deficiency and whose diarrhea clinically responded to treatment for Giardia. For E. histolytica, 15 of 16 (94%) patients infected with E. histolytica were positive by ELISA. 98 (92%) of 106 specimens from patients without demonstrable E. histolytica in their stool were negative by ELISA. Coinfection with multiple other parasites did not appear to affect sensitivity or specificity. These ELISA tests are simple, sensitive and specific diagnostic tests which will be useful both in individuals and in large scale epidemiologic investigations. Present studies are under way to develop this test for the detection of Cryptosporidia and Isospora antigen from AIDS and non-AIDS patients.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00360-01 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Reticuloendothelial Function in Patients with AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Thomas C. Quinn, LCI/NIAID

COOPERATING UNITS (if any)

Johns Hopkins University School of Medicine

LAB/BRANCH

Laboratory of Clinical Investigation

SECTION

Clinical Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

0.40

PROFESSIONAL:

0.4

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients with AIDS have repeated episodes of severe systemic infection associated with marked abnormalities in immune function. Perhaps the principle system for clearing the bloodstream of infection is termed the reticuloendothelial or mononuclear phagocyte system. In general, in systemic infections, this system shows enhanced capacity to clear the bloodstream of foreign substances. We have instituted a prospective study to examine alterations in RES function in homosexual men with acute self-limited viral infections, lymphadenopathy and AIDS. RES activity in patients is assessed by measuring F₅₁ and C3b receptor functions via clearance kinetics of IgG and IgM sensitized ⁵¹Cr-labeled erythrocytes. These studies will be performed serially on each patient and correlated with disease progression, immune complexes, RES blood flow kinetics, plasmaphoresis, and other immunologic alterations such as T cell responsiveness, subsets and Fc C3b receptors on circulating monocytes. In preliminary studies we have examined two AIDS patients with Kaposi's sarcoma, six AIDS patients with opportunistic infections, three lymphadenopathy patients (one on plasmaphoresis) and three "healthy" homosexual men. All but one of the "control" homosexual patients had elevated immune complexes (40-60 C1q binding assay). All the AIDS patients had prolonged clearance of IgG-sensitized RBCs (mean t-½ 98 min, slope 0.007) compared to normal or slightly accelerated clearance rates in the lymphadenopathy patients and controls (mean t-½ 28 min, slope 0.02). Two of the AIDS patients demonstrated prolongation of clearance rates with further deterioration in their clinical status suggesting further alteration in RES function. Similar results were found in a patient with Kaposi's sarcoma receiving interferon. These patients and others will be studied to assess alterations of RES function in relation to further disease progression, response to therapy and whether RES dysfunction predates the first opportunistic infection.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00361-01 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Alterations Which Correlate With Immunosuppression In AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Thomas C. Quinn, Senior Investigator, LCI/NIAID

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SECTION

Clinical Immunology

INSTITUTE AND LOCATION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	

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- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A common denominator of patients with the acquired immune deficiency syndrome (AIDS) appears to be a broad spectrum of immune deficiencies. However, it is clear that we continue to lack the full understanding of the mechanism (s) responsible for this immunologic dysfunction. We have initiated a study to examine whether peripheral blood lymphocytes from AIDS patients evidence a pattern of in vitro CMI response against mitogens and against specific viral antigens (CMV, HSV, BKV), in relation to disease progression, in vivo data generated by skin testing, and data generating by enumeration of T cell subsets as observed over a period of time. The ability to reconstitute the in vitro CMI response to specific viral antigens and mitogens will be measured by treatment with biologic modifiers such as IL-1, IL-2 and other lymphokines. Lastly, plasma from AIDS patients is examined to determine whether it contains immunosuppressive factors to normal T lymphocytes in the above assays, and whether these factors can be modified or detected through plasmaphoresis. In preliminary studies, 10 homosexual men with lymphadenopathy and 10 homosexual men with AIDS have been studied longitudinally. AIDS patients (8 with opportunistic infection and two with Kaposi's sarcoma) and all lymphadenopathy patients were skin test anergic, had reversed T helper/T suppressor cell ratios with prominent T helper cell depletion, and demonstrated hypergammaglobulinemia and elevated immune complexes. Healthy homosexual men and patients with lymphadenopathy responded to pokeweed mitogen and viral antigens in lymphocyte transformation studies and these responses were enhanced to control levels with the addition of interleukin-2. Significantly, lymphadenopathy patients lacked the ability to produce lymphokine-leukocyte migration inhibition factor (LIF) following antigen stimulation. This latter finding correlated with in vivo anergy in all instances. Patients with AIDS demonstrated a marked reduction in lymphocyte transformation response to pokeweed mitogen and a total absence of responsiveness to viral antigen. Lymphocyte transformation was partially restored by interleukin-2 in this latter instance. However, the ability to restore antigen induced responsiveness by IL-2 markedly decreased with disease progression, and lymphokine production remained negative. These data thus far demonstrate a gradient of immune dysfunction that develops in AIDS and further study is warranted in regards to the exact nature of the immunosuppressive factors and their effect on immune regulation as measured by lymphokine IL-1 and IL-2.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00379-01 LCI

PERIOD COVERED

October 1, 1982 to September 30, 1983

(Title of project (80 characters or less. Title must fit on one line between the borders.)

Studies of DNA Viruses and Other Possible Agents in AIDS Patients

PRINCIPAL INVESTIGATOR (*List other professional personnel on subsequent pages.*)*(Name, title, laboratory, and Institute affiliation)*

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

(Summary of work (Use standard unreduced type. Do not exceed the space provided.)

For the past two years this laboratory has been involved in extensive studies of herpes virus infections in AIDS patients. We have now developed techniques in this laboratory to expand our effort to assess AIDS patient specimens for conventional and fastidious adenoviruses as well as human papovaviruses. In addition, we will expand our preliminary efforts using established monocyte B cell and T cell lines to search for new AIDS-associated agents.

LABORATORY OF IMMUNOGENETICS
1983 Annual Report
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Annual Report
Laboratory of Immunogenetics
National Institute of Allergy and Infectious Diseases
October 1, 1982 to September 30, 1983

RESEARCH PROGRESS

The major subject of study within the Laboratory of Immunogenetics is the molecular anatomy of the immune system. This involves identification, isolation, structural and functional characterization of serum and cell-surface molecules. Techniques in immunology, protein chemistry, cell biology and molecular biology are brought to bear as appropriate to solve problems of interest. Systems currently under study include major histocompatibility complex (MHC) antigens and other lymphoid cell surface antigens of the human, mouse and rabbit, immunoglobulins of the human and rabbit and other immunologically relevant serum proteins. Some of the molecules under study have not previously been described or are poorly characterized structurally. For such molecules, structural study can uncover homologies to other better characterized molecules and define polymorphism and other important features. For well known molecules, studies are aimed at an understanding of functional or genetic regulatory aspects of the structure of the molecules themselves or the genes which encode them.

Human histocompatibility antigens: A monoclonal antibody (McAb 33.1) was raised in this laboratory previously by immunizing mice with a human EBV-transformed B lymphoblastoid cell line. Preliminary work suggested that McAb 33.1 recognized a B cell specific antigen (termed 33.1) which appeared to have a temporal association in its expression with B cell activation. Over the past two years, concentrated effort by several members of the laboratory (Kuo, Li, Sogn, Coligan, Marti) has yielded a great deal of structural, serologic and genetic information about 33.1 and permitted fairly precise placement of this antigen in the growing family of human class II MHC products.

Antigen 33.1 is poorly expressed on circulating B cells and appears not to be present on T cells or macrophages. It becomes much more strongly expressed on B cells after the B cells are activated by EBV infection or by B cell mitogens. After intrinsic radiolabeling of 33.1 by culture of a BLCL with ³H amino acids, the radiolabeled 33.1 can be isolated with McAb 33.1 and examined structurally. The antigen obtained in this manner was shown to have two chains generally similar to but definitely lower in molecular weight than the chains of the human class II HLA-DR antigens. This structural distinction, in combination with the uncharacteristic cellular distribution, indicated that McAb 33.1 was recognizing another human class II antigen. In order to test this possibility, detailed structural studies were initiated. Intrinsically radiolabeled 33.1 was isolated by immunoabsorbent chromatography and the chains were separated by preparative SDS-PAGE (Kuo, Li). Radiosequence analysis provided unambiguous proof that 33.1 is a non-DR human class II MHC antigen. It further showed that 33.1 can be placed in the DC (MB) family of class II molecules by virtue of its obvious homology to the murine I-A antigen. In a survey of partial sequence analysis of 33.1 from a large number of B cell lines, it was shown that the sequence obtained correlated with the MB type but minor structural and functional differences, which were magnified by analysis of the deletion mutants of Dr. R. DeMars, proved that 33.1 is distinct from the MB antigen. The exact interrelationship of 33.1 and MB remains to be determined.

As a complement to structural studies of 33.1 and other class II antigens, the serologic assay for class II allospecificities developed previously (Sachs, Kuo) has been useful. This is an inhibition assay based on the microlymphocytotoxic test and can be applied to cell lysates and purified fractions from cell lysates. Alloantisera for class I and class II MHC antigens have provided a great deal of genetic information but have not been useful for structural studies. By isolating molecules with xenogeneic monoclonal antibodies and examining the allospecificities of the isolated fractions, information from both types of antibodies can be correlated. It has been shown that material adherent to McAb 33.1 possesses some MB activity, but not all (Kuo, Li) whether this reflects cross-reactivity of 33.1 with MB, contamination of anti-MB sera with anti-33.1 or some other interaction is under study. Methods have been developed (Kuo, Li, Primerano) for the isolation of milligram amounts of 33.1, MB and DR from the same cell line. These methods have already in pilot experiments provided enough material for N-terminal sequencing of 33.1. The material isolated should be useful both for structure and for serology.

Another approach to understanding the organization, regulation and structure of human class II products is examination of the genes encoding them. A major resource for such studies has been created (Robinson) by obtaining lymphocytes from 55 members of seven families that have been identified serologically as including an individual inheriting a recombinant HLA haplotype. These lymphocytes have been transformed with EBV and DNA has been isolated from all of the cell lines. With such cell lines a number of experiments are possible. First, they are a very good source for isolating HLA antigens at the protein level. A major drawback to many structural studies has been that the cells used as a source of antigens were either of unknown HLA type or had been incorrectly typed. These cell lines have been typed directly and the genotypes are confirmed by family studies. Second, because of the presence of recombinant individuals, DNA from the cell lines may potentially be used to map antigens whose location is not currently known (class II and class III antigens). The easiest way to do such mapping is to find and follow a restriction fragment that is affected by recombination. Fragments of interest can be selectively visualized by hybridization with a large number of available probes (Long, Robinson). So far, it has not been possible to find informative restriction fragments, but these studies are continuing. It is not necessary to find such informative fragments, however, for successful use of these cell lines. Individual genes of interest will be cloned from the DNA and used for structural study. Ultimately, a physical map of all of the relevant genes will be constructed to replace the genetic maps now in use.

Studies are in progress with human class I antigens to identify amino acid residues involved in recognition by cytotoxic T cells (van Schravendijk, Coligan, Cowan). The HLA-A3 molecules from cell lines recognized as different by A3-restricted anti-influenza CTL's have been isolated. These molecules are very similar but small differences have been detected both in isoelectric points and in tryptic peptide maps. The nature of the differences is currently being studied by radiosequence methods. These experiments will provide the first level of information with regard to differences, but they are too cumbersome to provide all of the information desired. It is now possible to isolate and sequence the entire HLA-A3 gene from normal and variant individuals and such an approach is now underway to complement and extend the protein level results.

Other human cell-surface antigens: Studies with monoclonal antibodies are beginning to define molecules on T lymphocytes which are involved in specific T cell functions. Among these molecules, the antigens T3 and T8 are currently under study (Coligan) in collaboration with Dr. C. Terhorst. T3 is an ubiquitous T cell antigen and has been implicated in multiple T cell functions. It has been postulated to be an invariant portion of the T cell receptor for antigen. T8 is important for CTL recognition of class I MHC antigens. Partial N-terminal sequences of both antigens have been obtained by radiosequence methods. The goal of these studies is to identify contiguous stretches of amino acids suitable for construction of synthetic DNA probes useful for isolation of the genes encoding T3 and T8.

A second type of approach to the study of T cell surface molecules involves production of T cell hybridomas with defined functional properties. Human T cells with cytotoxic activity for human class I and class II antigens have been fused with mouse (Folks) and human (Jackson) T cell tumors to produce hybridomas. In early results, several mouse-human hybrids have been shown to retain some functional aspects of the human T cell parents. These cells are being characterized as to human and mouse surface antigens and are being evaluated as possible sources of antigen-specific receptor molecules for structural study.

Murine class I and class II MHC antigen. The extensive amino acid sequence data obtained in this laboratory on a number of murine class I molecules provide the necessary background for structural resolution of some interesting genetic observations and offer approaches to defining specific regions of MHC antigens involved in functional interactions among lymphocytes.

Previous results from this laboratory indicated the existence of three class I molecules (D, L and R) encoded within the D region of mice of the d and g haplotypes. The relationships among these closely linked products has been examined in the g haplotype (Lillehoj) by radiosequence methods. All of the molecules yielded identical patterns of CNBr fragments. N-terminal amino acid sequencing of these fragments distinguished H-2R^g from H-2L^g and H-2D^g but did not distinguish between the latter two. However, tryptic peptide maps using material labeled with ³H-Arg also revealed differences between H-2L^g and H-2D^g. The three antigens are therefore products of three distinct genes, but the similarities among them are striking. The degree of difference between H-2L^g and H-2D^g is of the same order as the differences found among the H-2K^b mutants. In addition H-2R^g, while somewhat more distinct, is more similar to H-2D^g and H-2L^g than to any other H-2 molecule yet sequenced. This suggests strongly that the D^g region has undergone recent expansion.

The amino acid sequence of the first 100 residues of the H-2K^k antigen has been completed. Six positions contain residues unique to the K^k molecule. The sequence obtained is somewhat different from that reported by others previously. Whether this reflects sequence errors or indicate the existence of multiple K^k antigens is not yet known.

Primary structural studies are also being done with murine class II antigens. Immunoprecipitation has suggested the existence of multiple I-E molecules in the d haplotype. Amino acid sequence studies are being done to determine whether the molecules are encoded by different genes (Maloy). The I-A antigen of wild mouse H-2 haplotype W12A is under study because of its close

serologic relationship with I-A^k. The two antigens are readily distinguishable functionally, so structural studies of these molecules should reveal some information about functionally relevant regions of the molecules which are not important for serologic reactivity.

Other experiments to elucidate the regions of murine (and human) MHC antigens crucial to allospecificities and specific functions involve the production of synthetic peptides (Maloy). Specific peptides which appear from comparative sequence studies to be important to the definition of a single MHC antigen are being synthesized and rabbit antisera are being prepared against the synthetic peptides. The antisera are tested for binding to the peptide and the intact antigen and for inhibition of cellular functions mediated by the antigen. Currently, 15 peptides from murine class I antigens have been prepared and antisera against them have been raised. In addition, a few peptides have been made corresponding to murine class II sequences. There is widespread interest in this new experimental approach and the availability of the peptide synthesis equipment and expertise has led to collaborative studies with other groups within the institute interested in a broad range of applications.

Rabbit MHC antigens. Previous structural studies of rabbit class I MHC antigens using monoclonal antibodies or anti-rabbit β_2 -microglobulin to isolate intrinsically radiolabeled molecules have now been extended dramatically by cloning and sequencing the genes encoding these antigens (Tykocinski). A size selected (16-17S mRNA) cDNA library was constructed using the recently described Okayama/Berg cloning method. This method maximizes the yield of full-length cDNA copies of the input mRNA. When the library had been prepared, it was screened by hybridization with a murine class I probe which was shown in preliminary experiments to cross-hybridize well with rabbit DNA sequences. A total of 37 colonies hybridizing strongly with this probe were selected and partial restriction maps of the 37 were done to determine the number of unique products cloned. Two of these clones, pR9 and pR26, were selected for sequencing. pR9 proved to be a full-length clone (1377 base pairs of insert) which was representative by mapping of most of the 37 clones obtained. pR26 was a rare clone and it contained an insert of 1169 base pairs which lacked the nucleotides encoding the first 18 N-terminal amino acids. Both clones contain all of the features expected for cDNA clones of class I antigens. The translated sequence of pR9 is identical to the N-terminal sequence obtained from the RL-5 cell line by radiosequence methods. The pR26 translated sequence is different at 4 of the 19 positions available for comparison. These results suggest that rabbits do have multiple class I antigens but that, at least in the RL-5 cell line, one antigen (represented by pR9) is predominant. At least one other antigen is expressed (represented by pR26) but at such a low level that it was not seen when unseparated class I heavy chain were subjected to sequence analysis. Comparison of the rabbit gene sequences to gene sequences from mouse and human has yielded new insights into class I gene evolution. Examination of rabbit class I gene organization at the genomic level is now in progress (Marche). A DNA library has been constructed and a clone from the library containing the pR9 genomic sequence is being studied.

Characterization of rabbit lymphocytes. The surface antigens and functional interactions of rabbit lymphocytes are under study using a panel of monoclonal antibodies prepared against a rabbit T cell tumor line. The antigens recognized by these antibodies have been characterized as to electrophoretic mobility (molecular weight), carbohydrate content, heat stability and cellular

distribution. Recently, primary structural studies have been initiated for several of these antigens. The most interesting antigen is L11/135, which appears to be present in large amounts on all T cells. It is a large glycoprotein (120,000 mol. wt.) and has as yet been refractory to sequence study. Experiments are under way to try to remove the presumptive N-terminal blocking group so that structural studies can be done.

Long-term continuous cultures of lines and clones of rabbit streptococcus-specific T cells are under study. In the last year an *in vitro* antibody restimulation assay for anti-streptococcal antibody has been developed to permit assessment of the helper or suppressor potential of these T cell lines and clones. Preliminary results have been encouraging. Establishment of function for these cells will facilitate evaluation of the possible functional properties of the antigens recognized by the panel of anti-T cell monoclonal antibodies.

Rabbit immunoglobulin allotypes. The group b allotypes of the rabbit C_k region are the most diverse group of genetic variants found in any species.^K Five group b allotypes are known in the domestic population and about an equal number have been described in wild rabbits. Several of these wild rabbit allotypes have now been studied at the gene and protein level. Allotypes b97 and b98 have been sequenced for approximately 60 of their 100 residues (Sogn). Despite considerable serologic differences, they are, in the region studied, the most similar pair of allotypes yet examined. In addition, they are most similar of all the allotypes to the sequence produced by selecting the most common residue at each position. The pattern of variability which is evident by adding these sequences to those already known argues strongly for gene conversion or a similar mechanism as the generator of rabbit allotype diversity.

Amino acid sequence analysis of the a-negative rabbit H chain of rabbit-mouse hybrid H89 (Sogn, Kuo) has revealed that the H chain is structurally identical in the amino terminal 20 residues to the known sequence for a2 H chains. Rabbit H chains belong to one of two well described subgroups. Most are of the a-positive subgroup, and all of these bear serologic determinants of the a1, a2 or a3 allotypes. Variants within these allotypes have been described, but none which is devoid of allotypic activity. Thus H89, which lacks all group a activity was expected to be of the a-negative subgroup. However, it bears no similarity to this subgroup by sequence. This paradox remains unanswered.

Immunoglobulin genes of rabbits and humans. Because of the diversity discussed briefly in the section above, the rabbit group b allotypes have long been hypothesized to be encoded by genes under unique control mechanisms. For this reason, some of the genes have now been cloned and sequenced. Initially, a rabbit-mouse hybridoma secreting a b4 rabbit k chain was used to obtain a cDNA clone (Dreher). This clone was sequenced and fragments of the clone were used as specific probes for V, J and C_k sequences in rabbit genomic DNA. Two genomic clones from C_k have now been obtained from a b4 rabbit sperm DNA library (Emorine, Esworthy). The first, called b4Nb4, has been identified as encoding the nominal b4 C_k region corresponding to the cDNA. The 5 Kb region containing C_k and 5 J genes has been sequenced. Only one of the 5 J genes appears to be functional^K. A second k gene (b4Ab4) has also been sequenced; it appears to include the C_k gene of the minor "K2" isotype. Although this gene is expressed at very low levels under normal circumstances, no major structural defects have

as yet been found in the b4Ab4 gene which would account for this. There is a deletion relative to b4Nb4 in the J-C intron but it appears not to involve the highly conserved potential regulatory "enhancer" region which has been described in this lab and others in the same general area. Studies to date have not solved the problem of latent allotypes. No simple genetic basis for this phenomenon has been found in the DNA samples examined to date, but a number of restriction fragments which hybridize weakly with a C_k probe have been identified and these remain as potential candidates.

The mechanisms underlying gene activation are being examined using human immunoglobulin genes (Max). The states of methylation of the kappa, mu, epsilon and J protein genes are being examined in normal and tumor cells representing the full range of states of immunoglobulin gene activation. Preliminary results indicate that the states of methylation do correlate well with rates of gene transcription in most cell lines examined.

Monoclonal antibodies to insulin. Studies designed to map the epitopes on insulin recognized by 18 monoclonal anti-insulin molecules have been completed (Schroer). Anti-idiotype antibodies have been prepared in rabbits to six of the antibodies which recognize different determinants on insulin. Of 13 anti-idiotype sera prepared, 11 recognized private idiotypes not present in nonimmune serum. Most of these recognized only the immunogen, although a few weak cross-reactions were noted. The two anti-idiotypes which reacted with something in normal sera were injected into mice. One of these sera stimulated Id-positive anti-insulin antibody in 9/86 mice receiving anti-idiotype in CFA and boosted. The other was without effect. This apparent immune network effect will be studied in more detail.

Functional studies of murine T cells. Two murine T cell hybridomas derived from ovalbumin-specific T cell blasts have been characterized (Folks). One hybridoma, OTH-6, produces T cell help for anti-ovalbumin antibody production in the presence of autologous antigen presenting cells, antigen and Con A supernatant. The other hybridoma, OHT-18, lacks antigen specificity but is capable of augmenting the helper capacity of suboptimal numbers of antigen-specific T cells in the presence of autologous cells. The functional activity of OHT-18 but not OTH-6 is carried by spent supernatant from the appropriately stimulated hybridoma.

In collaborative experiments with the Laboratory of Immunology, NIAID, two groups of insulin-specific T cell hybridomas were examined. The cells were derived from cells originally stimulated with pork insulin. By examining the specificity of the hybridoma response to insulin from different species, it was possible to show that the two groups of hybridomas were specific for distinct regions of the insulin molecule. All of the hybridomas were examined for anti-insulin idotypic specificities with the rabbit anti-idiotypic sera described above and for functional inhibition by monoclonal anti-insulin antibodies specific for a variety of insulin epitopes.

Hamster female protein. Hamster female protein (FP) is the hamster homologue of amyloid P component, an ubiquitous but minor component of all amyloid deposits (Sogn). In the hamster, FP has come under sex hormone control and is expressed at 100-1000 times the level in females that it is in males. This disproportionate expression appears not to harm the females. Partial sequence studies of FP at the protein level have revealed the close relationship

to amyloid P component but the complete structure has not been done because of the paucity of sites for useful chemical or enzymatic cleavage in FP. In order to complete the protein structure and to determine why FP is under hormonal control, mRNA has been prepared from the livers of male and female hamsters, translated in vitro to yield FP and cDNA prepared from the mRNA has been cloned by the Okayama-Berg method. Work is under way now to isolate clones containing FP cDNA.

Annual Report
Laboratory of Immunogenetics
National Institute of Allergy and Infectious Diseases
October 1, 1982 to September 30, 1983

Administrative Report

Within the period covered by this report a number of personnel changes have taken place in the Laboratory of Immunogenetics. Dr. Henry Krutzsch has joined the National Cancer Institute. Dr. Mei-chang Kuo will leave July 1 to join the Polaroid Corporation. Dr. Kevin Dreher has joined the faculty of the West Virginia University School of Medicine. Dr. Mark Tykocinski will join the staff of Case Western Reserve Medical School in July.

Dr. Thomas Kindt has spent this past year on sabbatical at the Institut Pasteur in Paris, France in the laboratory of Dr. Pierre-Andre Cazenave. He will be returning to the laboratory on September 1, 1983.

Dr. Patrice Marche joined the laboratory in March as a Visiting Fellow for one year. He has been assigned to the Immunogenetics Research Section of the laboratory. The laboratory has been joined by Dr. Steven Esworthy, a Staff Fellow from the National Institute of Mental Health and by Dr. Ronald Lieberman, a Medical Staff Fellow from the National Heart, Lung and Blood Institute. They are assigned to the Immunogenetics Research Section. Dr. Elliot Cowan, who received his Ph.D. from Washington University at St. Louis in June, has joined the Membrane Antigens Structure Section and Dr. Marilyn Lightfoote, who received her Ph.D. from University of Virginia in June, is now a member of the Immunobiology Section. Dr. Eric Long will join the laboratory in July. He has been on the staff of the University of Geneva.

Dr. W. Lee Maloy is still located in Building 8 and he will be moving to Building 5 as soon as renovations on his laboratory are complete. He has established a synthesis section at the Frederick Cancer Research Facility in Frederick, Maryland. A portion of the Immunobiology Section has been relocated to Building 10, ACRF.

Annual Report
Laboratory of Immunogenetics
National Institute of Allergy and Infectious Diseases
October 1, 1982 to September 30, 1983

Honors and Awards

Dr. Kindt has spent this year as a Visiting Scientist at the Institut Pasteur in Paris. He is working in the laboratory of Pierre-Andre Cazenave in the Immunology Department. Dr. Kindt has given seminars of laboratory work at the Institut Pasteur, at the University of Paris, at the Hospital Cochin, at the Institute for Immunology in Marseille, in Germany he has spoken at the Max-Planck Institute at Freiburg and at Tübingen. In addition, he has spoken at the Institute for Genetics in Cologne. Dr. Kindt has participated in teaching immunology at the Institut Pasteur and in addition, has given six lecture hours of instruction at the University of Tunis and gave a seminar of laboratory work at the University of Sfax in Tunisia. He has participated in doctoral examinations for a number of students both in Paris and in Marseille. Dr. Kindt has been invited to serve as a member of a review board for the American Cancer Society.

Dr. John Sogn was a visiting professor at Texas A & I in October 1982. He spoke at the Philadelphia Section, American Chemical Society in April, 1983. He was an invited speaker at the Second International Workshop on "Genetics of the Rabbit", March, 1983 and the New York Academy of Sciences meeting on "The Immune Network", in December, 1982. He was also a course lecturer on monoclonal antibodies at Catholic University in June 1983.

Dr. John Coligan was invited to give seminars at the University of Michigan and the Dana-Farber Cancer Center and has been invited to speak at the 5th International Congress of Immunology in Kyoto, Japan in August, 1983. He has been appointed as an editor of the Immunochemistry Section for the Journal of Immunology.

Dr. W. Lee Maloy was an invited speaker at the "H-2 Genetics Workshop" in Amsterdam in October 1982.

Drs. Marie Rose van Schravendijk and Erik Lillehoj were invited to speak at the FASEB minisymposia in April 1983.

Dr. Susan Jackson was invited to give seminars at Abbott Laboratories, in Chicago, February 1983 and at Catholic University, in February and June, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00166-06 LIG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Rabbit MHC Genes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Thomas J. Kindt, Chief, LIG, NIAID

COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.2	0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A single rabbit class I MHC (RLA) heavy chain has been detected in the rabbit lymphoid cell line RL-5 using conventional class I immunoprecipitation methods (anti-Beta-2-microglobulin; monoclonal antibodies). To investigate RLA structure and expression at the nucleic acid level, an Okayama/Berg cDNA library was constructed from size-selected RL-5 poly-A mRNA, and pR9, a full-length cDNA clone, encoding this RLA heavy chain, was identified. pR26, a cDNA clone distinct from pR9, encodes a class I MHC protein product not detected in RL-5 cells. To study the genomic class I locus, λ 19-1, a genomic clone isolated from an RL-5 λ J1 library and encoding the pR9 mRNA, is currently being sequenced. These studies employing recombinant DNA techniques will be extended to further elucidate RLA gene organization and expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00168-06 LIG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoglobulin Allotypes of Heavy and Light Chains

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Thomas J. Kindt, Laboratory Chief, LIG, NIAID

COOPERATING UNITS (if any)

P. A. Cazenave, Institut Pasteur, Paris, France and Amel Benammar, University of Tunis, Tunisia

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetic Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MAN YEARS:

0.4

PROFESSIONAL:

0.4

OTHER:

.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Structural and genetic studies are in progress on kappa chain allotypes of wild rabbits. The kappa chain allotypes are so diverse in rabbits that it is of interest to determine the mechanism by which the allotypes have diverged. Hypotheses generated by examination of domestic rabbits are being tested using the additional data available from new wild rabbit allotypes which have been bred onto domestic rabbit backgrounds. Most of the amino acid sequence of allotypes b97 and b98 have been determined and nucleic acid probes for kappa chain J and C regions have been used to examine the kappa chain genes of both wild and domestic rabbit allotypes. These studies indicate similar gene arrangements and suggest gene conversion as a mechanism for allotype divergence.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00169-06 LIG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Primary Structural Analysis of Murine and Human Transplantation Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

John E. Coligan, Research Chemist, LIG, NIAID

COOPERATING UNITS (if any)

David Sachs, NCI, NIH; S. Shaw, NCI, NIH; B. Biddison, NINCDS; Ted Hansen, Washington University at St. Louis, Edward Wakeland, University of Florida.

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Membrane Antigen Structure

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.1	2.5	1.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Human and murine class I and class II molecules are isolated and their primary structure analyzed. The goal of these studies is to gain an understanding of their function and antigenic properties in molecular terms as well as to gain knowledge of their evolutionary relationships. Structural analyses of D region molecules of the g haplotype have shown the existence of three unique gene-products - D^d, L^d and R^d. These molecules are more closely related than any previously described H-2 molecules suggesting that they have arisen by relatively recent gene duplications. In addition, extensive amino acid sequence information has been acquired for the H-2K molecule allowing comparison to previously determined sequences of other class I molecules. In the case of human class I molecules, the location of structural differences in HLA-A3 molecules recognized as being different by cytotoxic T cells is in progress both at the protein and gene level. Studies on murine Ia molecules are seeking to prove whether or not two structurally distinct IE molecules are present in the mouse. In addition, the nature of the structural differences between wild type IA molecules (Ia^{KW12a}) and IA molecules are being determined. These molecules are virtually indistinguishable serologically, but are readily distinguishable by MLR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00170-06 LIG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Human Histocompatibility Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Eric Long, Expert, LIG, NIAID

COOPERATING UNITS (if any)

John Sachs, University of London and A. H. Johnson, Georgetown University

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
1.85 1.45 0.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An assay based on inhibition of microlymphocytotoxicity has been developed for monitoring purification of HLA-encoded antigens. Normal methods are not applicable to cell lysates of purified fractions so limited information is available on allospecificities of isolated molecules. The new assay has been successfully used to shed light on the interrelationships of monoclonal antibodies and alloantisera for class II MHC antigens, particularly 33.1.

The map order of genes within the HLA complex were analyzed by studying genomic DNA restriction enzyme polymorphisms in seven families that were known to include individuals inheriting recombinant HLA haplotypes. Cloned DNA for class I, class II, (DR α , DR β , DC α , DC β and SB β) and class III (C4) MHC genes were used as probes in Southern blotting analysis. Polymorphism in the size of restriction fragments from the various MHC gene were observed with certain enzymes and could be assigned to haplotypes in the families. The results to date indicate that the genomic restriction enzyme polymorphisms correlate with the assignments made by classical typing techniques.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00171-06 LIG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Studies on Rabbit Immunoglobulins and Other Serum Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

John A. Sogn, Chemist, LIG, NIAID

COOPERATING UNITS (if any)

T. Chused, LMI, NIAID and J. Coe, RML, NIAID

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.3

PROFESSIONAL:

1.1

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The set of monoclonal antibodies prepared against rabbit lymphocytes has been used in two separate types of experiments. The cell-surface molecules recognized by these antibodies have been isolated and primary structural studies have begun on several of them. In addition, their ability to recognize cell subpopulations have been useful both in preparative separation of normal rabbit lymphocytes and in functional analysis of rabbit T cell lines and clones specific for streptococcal antigens. An ELISA for anti-streptococcal antibody has been developed to facilitate characterization of these rabbit T cell lines. In separate experiments, hamster female protein, an acute phase protein under sex hormone control has been characterized by primary structural analysis at the protein level and preliminary studies at the DNA level. Messenger RNA has been prepared and cloned in an effort to complete the structural studies and to understand the hormonal regulation of this gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00172-06 LIG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Synthesis of Peptides

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Walter Lee Maloy, Expert, LIG, NIAID

COOPERATING UNITS (if any)

Edward Wakeland, University of Florida; David Sachs, NCI; Gilbert Jay, NCI.
Malcolm Martin, LMM, NIAID

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Membrane Antigen Structure

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.3

PROFESSIONAL:

1.1

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Through protein and DNA sequence analysis, much information on the primary structure of proteins has accumulated over the past several years. We have used this information to prepare antibodies reactive with defined portions of proteins. This is done by synthesizing 10-30 residue peptides that correspond to sections of the sequence of the protein. After coupling to a protein carrier, antisera to the peptides are prepared. In the case of transplantation and other lymphocyte antigens, these antibodies will be used to define sites of cellular recognition and serological specificities. In the case of the viral antigens the antisera will be used as specific probes to detect the expression of endogenous retroviral DNA found in human cells or as antisera specific for xenotropic murine leukemia virus or murine mink cell focusing virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00173-06 LIG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control of Gene Expression

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Edward E. Max, Commissioned Officer, Laboratory of Immunogenetics, NIAID

COOPERATING UNITS (if any)

Dr. Stanley Korsmeyer, NCI, NIH

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

4.2

PROFESSIONAL:

3.3

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are exploring gene regulation of rabbit κ immunoglobulin genes by characterizing several genomic clones related to this system. The important regions of the nominal b4 allotype κ gene have been sequenced (about 5 Kb including complete J-C locus). Other C genes currently under investigation include a gene from b4 DNA encoding the κ 2 isotype and a gene apparently encoding the nominal b5 allotype κ chain. In addition, we are sequencing a germline genomic V region clone and an enigmatic rearranged gene isolated from a rabbit-mouse hybridoma. Probes from cloned genomic DNA have been used to characterize, by Southern blot analysis, κ -related sequences in DNA from rabbits of several b series strains.

We are also studying the expression of four human genes whose activity is regulated in B cell development. In collaboration with Dr. Stanley Korsmeyer of NCI, several leukemia and lymphoma lines representing B-lineage cells at different stages of differentiation have been examined for the methylation status of four genes; the methylation analysis is being correlated with the magnitude of gene expression as assessed by Northern blot analysis. These studies represent assessment of baseline parameters which we hope to be able to alter by in vitro manipulation of expression of these genes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00180-05 LIG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interspecies and Human-Human Hybridomas and Monoclonal Antibodies

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

John A. Sogn, Chemist, LIG, NIAID

COOPERATING UNITS (if any)

G. E. Marti, CC, NIH and R. DeMars, University of Wisconsin

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunogenetics Research Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

4.3

PROFESSIONAL:

2.9

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Monoclonal antibody 33.1, prepared against a human EBV-transformed B cell line has been characterized in detail. The antigen 33.1 is a human homologue of the I-A murine class II MHC antigen. The antigen has been characterized by immunofluorescence, flow cytometry, electrophoresis and primary structure analysis using radiosequence methodology. The results show that 33.1 is an I-A homologue but that it is distinct from the previously reported I-A homologues MB and DS. Unlike these antigens, 33.1 is virtually absent on resting B cells but strongly positive on activated B cells. It is very similar to MB in structure but can be distinguished by a few residues near the N-terminus.

Rabbit-mouse hybridoma H89 secretes an allotype-deficient rabbit H chain which nonetheless has all of the structural features of a H chain from the a-positive subgroup. Monoclonal antibodies have been prepared against human IgE.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00191-05 LIG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoregulation of T Lymphocytes by Immune Complexes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Kenneth W. Sell, Section Head, LIG, NIAID

COOPERATING UNITS (if any)

Ethan Shevach, LI, NIAID

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Immunobiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.8

PROFESSIONAL:

1.8

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Murine T cell hybridomas for ovalbumin (OVA) and insulin have been produced and tested for ability to regulate the responses of specific B cells. Several categories of T cell hybridomas have been described. Some kinds of T cell hybridomas secrete IL-2 specifically when stimulated with the correct antigen, insulin or OVA, and in addition, help OVA-specific histocompatible B cells or TNP-specific histocompatible B cells make antibodies while others do not help specific B cells make antibodies. Other T cell hybridomas are self reactive but not antigen specific and these augment suboptimal numbers of antigen primed T cells in B cell secretion assays. For the insulin system, B cell hybridoma antibodies have been used to prepare anti-idiotypic reagents that do not affect IL-2 secretion by any anti-insulin-specific T cell hybridomas studied to-date. One of these anti-idiotypes clearly does produce regulatory effects in an insulin immune response when examined in in vivo or in vitro assays. Future work will involve further experiments to define cellular control of the network activated by this anti-idiotype. In other recently initiated experiments, human-mouse T cell hybrids are being used to study new human surface molecules at both the protein and gene levels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00352-01 LIG

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification and Structural Analyses of Human T-Cell Receptor Molecules

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

John E. Coligan, Research Chemist, LIG, NIAID

COOPERATING UNITS (if any)

S. Shaw, NCI, NIH; W. Biddison, NINCDS, NIH; and C. Terhorst, Dana-Farber Cancer Center

LAB/BRANCH

Laboratory of Immunogenetics

SECTION

Membrane Antigen Structure

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Various membrane-bound receptor molecules are undoubtedly important in the regulation of the immune response by T-lymphocytes. The goal of these studies is to identify and structurally characterize these receptor molecules and the ligands with which they interact. Project areas include: (1) studies on the primary structure of (glyco)proteins associated with the T3 and T8 complexes, (2) identification of the target antigens of two human cytotoxic T cell clones specific for class II molecules and (3) attempts to obtain a stable, quantitative source of homogeneous T-cell receptors by preparing T-cell hybridomas.

LABORATORY OF IMMUNOLOGY
1983 Annual Report
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PHS-NIH
Summary Statement
Office of the Chief
Laboratory of Immunology
October 1, 1982 through September 30, 1983

Introduction

The Laboratory of Immunology is concerned with the elucidation of the fundamental mechanisms underlying immunologic responses. It has made rapid progress through the use of three new technologies which are creating a revolution in immunologic sciences. These are the use of monoclonal antibodies, the adaptation of techniques of molecular genetics to immunologic problems, and the use of long-term lines of cloned normal and transformed lymphocytes. The continued use and major improvement of these approaches should allow solution of many of the major problems which have concerned immunologists and should provide important approaches to the more precise regulation of the normal and the disordered immune response.

An X-linked gene family expressed in lymphocytes.

Laboratory of Immunology scientists have developed an approach to the creation of complementary DNA probes that recognize genes uniquely expressed in the messenger RNA of one but not another type of lymphocyte. For example, B cell specific cDNA probes have been produced by exhaustive hybridization of B cell cDNA with an excess of messenger RNA prepared from a T cell tumor. This B cell specific cDNA hybridizes to approximately 90% with messenger RNA derived from a B lymphoma. By contrast, the B cell specific cDNA will hybridize to less than 10% with messenger RNA from a T lymphoma. This approach may also be used to create cDNA cloned libraries of B cell specific and T cell specific genes. When a cDNA specific for T helper, but not T suppressor, cells was used to probe a "T cell specific" cDNA library, a limited number of clones were identified. Amongst these was one which upon Southern blotting proved to recognize a large number of distinct restriction fragments. By hybridizing this cDNA probe to the DNA of somatic cell hybrids which contained only a limited number of mouse chromosomes, it was shown that virtually all of these restriction fragments were on the X chromosome. This suggested that the cDNA probe identified a family of X chromosomal genes which might be uniquely expressed within lymphocytes. Northern analysis revealed that four distinct species of messenger RNA were found in a series of T hybridomas and in the AKR thymoma line, BW5147. When B cell tumors were analyzed, the results were particularly interesting. Abelson transformed B cell lines failed to contain messenger RNA which hybridized with the cDNA probe in question. Similarly, the vast majority of B lymphomas of mature stage were also negative. However, a small number of B lymphomas, which appear to represent pre-secretory cells, do express messenger RNAs, distinct in their mobilities from those of T cells, which hybridize with the cDNA probe. Even more interestingly, a series of plasmacytomas also express messenger RNAs which hybridize with the cDNA probe; these messenger RNAs are distinct from those expressed in the pre-secretory lymphomas. Analysis of congenic strains created by transferring the xid genetic defect from CBA/N mice onto other backgrounds, such as MRL, indicates that genetic polymorphisms

identified in genes which hybridize with the cDNA probe are linked to the xid gene, which is responsible for the X-linked immunodeficiency. This raises the possibility that the xid gene may in fact be a member of this X-linked gene family. Detailed analysis of distinct cDNA clones, in both B cell and T cell libraries obtained from this family, are now in progress in an effort to get greater insight into the structure of these genes, the organization of the gene family and into the nature of their products (Cohen, Hedrick, Paul and Davis, LI, NIAID)

Molecular genetic analysis of rabbit immunoglobulin.

Rabbit immunoglobulins express several phenomena of particular interest in the understanding of the regulation of immunoglobulin gene expression. Thus, there are expressed in rabbits allotypic determinants which are found on the variable region of immunoglobulin heavy chains and which are inherited in a normal Mendelian dominant fashion. Secondly, many of the allotypic determinants expressed on heavy chain variable regions and light chain constant regions have been reported to be subject to a phenomenon known as latent or hidden allotyping in which an immunoglobulin allotype not expected to be expressed in a given rabbit because of its genotype is nonetheless found under certain unusual circumstances. The understanding of these two phenomena should provide a major insight into the regulatory processes which control Ig gene expression. Laboratory of Immunology scientists have had a long standing interest in utilizing the rabbit model for this purpose. To carry this out, rabbit immunoglobulin messenger RNAs of defined genetic origin have been isolated and complementary DNA probes have been constructed and sequenced. These have been used to study the expressed messenger RNAs and genomic DNAs prepared from genetically defined rabbits. Clones carrying cDNAs and coding for κ light chains of the B9 and bas allotypes, as well as the heavy chain VHA1, VHA2, $\gamma 12,14$ and $\mu 80$ allotypes have been constructed, identified and sequenced. The cDNAs complementary to the B4, B5, B9 and bas allelic type show extremely high conservation of their 3' untranslated regions (93 to 96%). This high conservation of 3' untranslated regions enables an approach to detect κ light chain messenger RNAs from rabbits of different κ allotypes in both northern and dot blot analysis. In addition, results from the sequence of cDNAs complementary to κ chain messenger RNAs has allowed the preparation and characterization of allotype specific probes. These probes now available will make possible an examination of messenger RNA produced by cells purported to produce latent allotypes either in culture or upon collection from living animals. These should allow a definitive understanding of the process resulting in latent allotyping. Efforts are also in progress to develop VHA allotype specific probes which should be critical in an examination of the mechanisms regulating A locus allotypy (Bernstein, Alexander and Mage, LI, NIAID).

Genetic control of antibody responses to phosphorylcholine

The immunoglobulin genes responsible for the production of antibody to phosphorylcholine are known with a substantial degree of precision. Most inbred strains of mice produce anti-phosphorylcholine (Pc) antibodies of limited heterogeneity involving three dominant light chain, heavy chain pairs. Recently, a population of wild mice, designated CNB, have been identified which fail to make any detectable anti Pc-antibodies upon immunization with a

wide range of Pc containing antigens. CNB mice were crossed to C.B20 mice and an F₂ generation prepared. Among those animals homozygous for Ig allotype genes of CNB, essentially none responded to Pc antigen. Of the remainder, approximately 50% were responders and 50% non-responders. These results indicate that CNB mice are unresponsive to Pc antigens because of the action of two distinct sets of genes. One set of genes linked to the immunoglobulin heavy chain allotypes is incapable of supporting response to Pc when it is present in the homozygous condition; the second set of genes acts dominantly, suggesting that their products are regulatory and prevent the response of mice to Pc . Since this gene segregates independently of the allotype, it seems possible that this gene is not directly associated with specifying an immunoglobulin region itself. More detailed analysis of this system should provide one of the most completely understood genetic systems for the analysis of those events important in determining whether antibodies can be raised against certain immunogens (Lieberman, Nishinarita and Humphrey, LI, NIAID; D'Hoostelaere and Potter, Laboratory of Cell Biology, NCI).

Molecular genetic analysis of class II major histocompatibility complex genes

Class II MHC molecules are heterodimeric cell surface glycoproteins expressed predominantly on B lymphocytes and macrophages. They show extensive intraspecies polymorphisms which are believed to reside principally in sequence differences between the constituent chains of the various mouse haplotypes. These polymorphisms are associated with two major immunological phenomena, the restriction of T cell interaction with antigen presenting cells and B cells, and immune response gene function. Laboratory of Immunology scientists wish to gain a thorough understanding of the structural details of class II molecules and, in particular, of the regions which specify the immunologically relevant polymorphic sites. The first step in the proposed program has involved the cloning of genes for class II MHC polypeptide chains. A cDNA clone corresponding to the 3' terminal 464 base pairs of the A_d^d messenger RNA has been obtained and completely sequenced. When used as a radioactive probe of Southern blots of mouse DNA from strains of various H-2 haplotypes, this cDNA reacts with a limited number of restriction fragments corresponding to what would be anticipated for a single copy gene. This single copy gene is surrounded by extensive restriction site polymorphisms in the various H-2 haplotypes. The cDNA clone has also been used as a probe for the isolation of complete genomic clones corresponding to the A_d^d and A_k^k genes. Such lambda genomic clones show all the restriction fragments seen in genomic Southern blots. This is consistent with the probe reacting with a single copy gene. Structural analysis indicates that the major site of polymorphic variation of these class II genes is in the 90 amino acids of the N terminal or β_1 domain of the β chain. A complete analysis of the functional properties of the class II genes would be enormously aided by the transfer of these genes to other cells so that site directed mutagenesis could be utilized as a tool for studying structure-function relationships. The genomic lambda clones of both A_d^d and A_k^k have been used to prepare high molecular weight DNA for cotransformation of LTK cells with the herpes TK gene, using the calcium phosphate precipitation method. A number of transformants have been isolated which, on Southern blotting, indicate the successful introduction of both the A_d^d and A_k^k gene. Northern blots of total RNA prepared from these same cells show significant production of A_d^d messenger RNA in both the A_d^d and the A_k^k gene transfer recipients. These results indicate that it is feasible to introduce

these genes and to determine their expression. The next requirement will be to introduce these genes into cells which are capable of using them in a functionally meaningful manner so that changes in the structure of the gene and in antigen presenting function may be correlated. To this end, efforts to introduce class II genes into B lymphoma cell lines are now in progress (Germain, LI, NIAID; Robinson and Margulies, National Institute of Child Health and Human Development; Seidman, Harvard Medical School).

Mutational analysis of the function of class II major histocompatibility complex molecules.

Class II major histocompatibility complex molecules, known in the mouse as Ia molecules, act as restriction elements in interactions of T cells with antigen presenting cells and B cells and are the products of specific immune response genes. Their function as restriction elements is dependent upon the ability of T cells to co-recognize them with antigens and on a requirement for such co-recognition in order for histocompatibility restricted T cells to become activated. The role of class II molecules as immune response gene products depends both on this property and upon the postulated property that these molecules bear a site which interacts with specific antigens, determining whether such antigens may be successfully presented to the T cells of an individual animal. An understanding of how class II molecules mediate these functions will require both a detailed structural analysis of the class II molecules and, more particularly, a determination of how variation in their structure changes their capacity to mediate specific antigen presenting and immune response gene determined activities. In order to begin this analysis, preparation of a series of class II mutants has been undertaken by Laboratory of Immunology scientists. A B cell-B lymphoma hybridoma, TA3, with potent antigen presenting activity was exposed to ethylmethane sulphonate, a mutagen, in order to induce mutations which could then be selected. Since this cell was heterozygous for the I-A^k gene, it was hoped that structural mutants might be obtained. Cells which survived the mutagenesis process were then exposed to a monoclonal antibody to the I-A^k encoded class II molecule, together with complement. This resulted in the cytolysis of approximately 99% of the cells in the culture. The remaining cells were grown up and then stained with a second monoclonal antibody also specific for the I-A^k encoded molecule but directed against a distinct determinant upon that molecule. Cells which expressed this second determinant were selected by electronic cell sorting and subsequent cloning. In practice, obtaining a reasonable yield of mutant cells required two rounds of cell sorting and two rounds of cytolysis with antibody and complement. Two general types of mutants were produced in this way. Type A mutants were selected initially by the utilization of the monoclonal antibody 10.2.16 and complement as the cytolytic agent and the monoclonal antibody 26.7.11 as the positively selecting agent, using electronic cell sorting. Type B mutants were selected in the opposite manner. Type A mutants were similar to one another serologically in that they failed to express antigenic determinants on the I-A^k molecule recognized both by a group of monoclonal antibodies but did express certain other antigenic determinants recognized by other monoclonal antibodies which are I-A^k specific. Current analysis strongly suggests that the change which has occurred in the type A mutants involves the I-A_B chain. The nature of this change has not yet been established. Both chemical and molecular genetic studies are in progress in an effort to determine this. Functionally, the

type A mutants appear to be of two general types. All of the type A mutants are unable to present antigen to one group of T cell hybridomas which utilize I-A^K antigens as restriction elements; similarly, all of the type A mutants are successful in presenting antigens to a second group of T cell hybridomas that also utilize I-A antigens as restriction elements. There are, however, a third group of T cell hybridomas which are able to recognize antigen presented by some, but not other type A mutants. This strongly suggests that at least two distinct events have occurred and that there are at least two different types of type A mutants. The type B mutants appear to have had a genetic change involving their I-A^K chains. They fail to express any antigenic determinant which appears to be dependent upon the I-A^K α chain for its expression. Thus far, none of the type B mutants has been successful in presenting antigen to any T cell hybridoma that utilizes the I-A^K molecule as a restriction element. A chemical and genetic analysis of these mutants is also planned. It is anticipated that such analyses will reveal important elements in the structural basis of the antigen presentation and Ir gene function mediated by class II molecules (Glimcher, Massachusetts General Hospital; Paul and Germain, LI, NIAID; Aszofsky, LMI, NIAID; Sharow and Sachs, Immunology Branch, NCI).

Induction of self tolerance can explain certain forms of immune response gene controlled responsiveness.

The immune response to thymus dependent antigens depends upon the activation of a series of immune response (Ir) genes which encode class II major histocompatibility complex molecules that are co-recognized with antigen by histocompatibility-restricted T cells. Strain 13 guinea pigs possess an Ir gene which allows them to respond to antigenic determinants on the B chain of bovine insulin. By contrast, strain 2 guinea pigs are unresponsive to these antigenic determinants. Laboratory of Immunology scientists have attempted to gain insight into the mechanisms responsible for this genetically controlled difference in responsiveness to insulin B chain determinants. They have done so by the induction of long term T cell lines from strain 13 guinea pigs specific for insulin B chain determinants. Long term T cell lines prepared from strain 13 T cells primed in vitro with insulin presented to them on strain 13 antigen presenting cells express the specificity normally associated with T cells from strain 13 animals immunized with insulin. That is, these T cells respond to the insulin B chain antigenic determinants when presented by strain 13 antigen presenting cells but not when presented by strain 2 antigen presenting cells. However, it is possible to prime strain 13 T cells which can respond to insulin when presented by strain 2 antigen presenting cells. This process takes advantage of a technique described several years ago by Drs. David Thomas and Ethan Shevach in the Laboratory of Immunology which involves the initial elimination of alloreactive T cells through the use of bromodeoxyuridine and light. The strain 13 T cell lines obtained, which respond to insulin presented by strain 2 antigen presenting cells, have several unique features. Firstly, they fail to react to insulin presented by strain 13 antigen presenting cells indicating that they utilize strain 2 class II molecules as restriction elements. Secondly, they recognize the insulin B chain in association with strain 2 antigen presenting cells. This is of particular significance since strain 2 guinea pigs failed to make any responses specific for insulin B chain determinants. Consequently, the unresponsiveness of strain 2 guinea pigs cannot be due to a total incapacity

of strain 2 antigen presenting cells to present insulin B chain. Finally, the strain 13 T cell lines which recognize insulin B chain on strain 2 antigen presenting cells react equally well to bovine insulin and to guinea pig insulin. This strongly suggests that strain 2 guinea pigs are unresponsive to insulin B chain presented by strain 2 antigen presenting cells because the T cell clones which might have reacted to it cross-react with self-insulin presented by strain 2 antigen presenting cells and, thus, would have been eliminated during the induction of self tolerance. This tolerance induction, however, fails to eliminate T cell specific for insulin B chain associated with strain 13 antigen presenting cells. These results thus indicate that the process of tolerance induction to T cells is itself restricted by the major histocompatibility complex; and secondly, that the T cell receptor repertoire is shaped in the course of tolerance induction in such a way as to explain at least certain instances of Ir gene controlled unresponsiveness. Finally, these results indicate that in such cases, one cannot attribute unresponsiveness dependent on Ir genes to a total incapacity of the antigen presenting cell to process or present the antigen in question (Dos Reis and Shevach, LI, NIAID).

Monoclonal antibody specific for receptors of MHC-restricted T cell clones.

One of the major problems facing cellular immunologists has been the nature of the antigen binding receptor of T cells and, in particular, the nature of the recognition structures involved in the phenomena of histocompatibility restricted T cell interaction with antigen presenting cells and B cells. The current availability of cloned long term T cell lines and T cell hybridomas that are antigen specific has made possible more direct approaches to the study of this problem than was formerly feasible. Laboratory of Immunology scientists have now produced monoclonal antibodies which appear to be specific for unique antigenic determinants associated with the antigen specific receptors of such cloned T cell hybridomas. These experiments involve the derivation of T cell hybridomas from B10A T cells specific for the carboxy terminal peptide of pigeon cytochrome c. Antibodies were produced by immunizing (BALB/c x AKR)F₁ mice with one such T cell clone, 2B4. Monoclonal antibodies were derived which had the property that they inhibited the response of 2B4 to pigeon cytochrome c as measured by the failure of 2B4 to secrete interleukin 2 in the presence of cytochrome c when the monoclonal antibody was present. A closely related hybridoma, 2C2, also specific for the carboxy terminal fragment of pigeon cytochrome c failed to be inhibited in its activation by the monoclonal antibody, indicating a high degree of specificity in the action of the antibody. This monoclonal antibody bound to 2B4 but not 2C2 cells as demonstrated by flow microfluorometry. The molecule on the surface of 2B4 which was recognized by the monoclonal antibody was a heterodimer consisting of two disulfide linked polypeptides, one of approximately 42,000 daltons, and the other, 48,000 daltons. The monoclonal antibody failed to precipitate such a molecule from 2C2 cells. Additional studies aimed at isolating and biochemically characterizing this 85-90,000 dalton heterodimer are now underway. It seems very likely that this molecule will prove to have the antigen binding properties of the T cell receptor. In addition, efforts to molecularly clone the genes which encode this molecule are also being undertaken (Samelson, Germain, and Schwartz, LI, NIAID).

Receptor occupancy model for T cell activation

The availability of cloned long term lines of T cells makes possible a detailed analysis of their requirements for activation. In particular, it is possible to determine both the characteristics of the interaction of the receptor with antigen on the surface of antigen presenting cells and what fraction of receptors need to be occupied in order for an activation event to occur. To begin this analysis, Laboratory of Immunology scientists have examined the activation requirements of T cell clones propagated *in vitro* by stimulation with antigen in association with class II MHC molecules on APC. Such lines are "rested" for various periods of time, in the absence of antigen, and then examined. T cells tested shortly after they have been placed into the rest cycle require as much as one hundredfold greater concentrations of antigen for their activation than do T cells tested after a period of 14 days during the rest period. An analysis of the mechanism underlying this phenomenon has been undertaken using approaches similar to those applied to the activation of cells by hormones. The results obtained indicate that T cells obtained after long periods of rest appear to compete much more efficiently for antigen than do T cells obtained shortly after the initiation of rest, suggesting they have either more receptors on their surface or these receptors have higher affinity for antigen. By contrast there appear to be no obvious differences in the innate sensitivity of these cells to interleukin 2, the T cell growth "hormone." The availability of reagents capable of recognizing antigen-specific receptors on the membrane will now make possible a direct examination of this dynamic process by techniques that are independent of the need for antigen binding itself (Ashwell and Schwartz, LI, NIAID).

Ultraviolet irradiation induces the synthesis of interleukin-1 by macrophages and epidermal cells.

There has been much recent interest in the effect of ultraviolet irradiation on normal immunologic function. Certain skin cancers arise after prolonged exposure to ultraviolet irradiation and defects in immunological function, principally increased suppressor T cell activity, are apparent after shorter periods of exposure to ultraviolet light. It has been reported that ultraviolet light treated macrophages are incapable of subsequent stimulation by various stimulants to produce factors such as interleukin-1. However, when the direct effect of ultraviolet irradiation on macrophages and epidermal cells was evaluated, it was found that there was a dose dependent increase, not inhibition, in both intracellular and extracellular interleukin-1 production by both macrophages such as the P388D1 cell line and epidermal cells. An analysis of the factor produced by UV irradiated P388D1 cells indicated that it had a molecular weight of 15,000 daltons which is that of authentic interleukin-1. Furthermore, the production of this UV induced interleukin-1 was completely inhibited by cycloheximide suggesting that ultraviolet light initiated the *de novo* synthesis of interleukin-1 rather than the death of cells with subsequent leakage of previously produced interleukin-1. Since UV induced interleukin-1 preparations are not contaminated with phorbol esters, they provide valuable tools for checking the properties of other interleukin-1 preparations. This has made possible a verification of the action of purified interleukin-1 on B cell activation utilizing highly purified B cells cultured at low density with anti- μ and B cell growth factor.

Under these conditions, both conventionally induced interleukin-1 and interleukin-1 induced by ultraviolet irradiation appeared to able to synergize with the other stimulants to cause B cell proliferation. The observation that ultraviolet light induces interleukin-1 production, particularly at low doses of irradiation, should make possible the greater understanding of the immunological consequences of such irradiation (Ansel and Green, LI, NIAID).

Monoclonal antibody to the IL-2 receptor complex

Interleukin-2 (IL-2) is a glycoprotein of 15,000 daltons secreted by T lymphocytes after stimulation with antigens and mitogens. IL-2 functions as a hormone -like growth factor for the proliferation of many subsets of T cells. It exerts its growth promoting properties after interacting with specific membrane binding sites expressed on activated by not resting T cells. These sites are specific for interleukin-2 and may be designated interleukin-2 receptors. Laboratory of Immunology scientists have produced rat monoclonal antibodies which appear to bind to the interleukin-2 receptor itself or to proteins closely related to that receptor. These antibodies were produced by immunizing rats with long term T cell lines which expressed interleukin-2 receptors. The monoclonal antibodies which were candidates for being specific for the receptor were identified because of their ability to inhibit the interleukin-2 dependent stimulation of activation of these lines. The antibodies obtained that appear to be specific for the interleukin-2 receptor have the property that they bind in high density to HT-2 cells and to T cell blasts induced by concanavalin A but not to resting T cells nor to non-activated thymocytes or to resting T cells. B cell blasts induced with lipopolysaccharide appear to express the antigen recognized by the monoclonal antibodies but at substantially lower density than HT-2 cells or T cell blasts. Although the monoclonal antibody studied in the greatest detail inhibits T cell activation, it does not block the binding of ^{3}H -IL-2 to HT-2 cells. However, if ^{3}H -IL-2 is allowed to bind to HT-2 cells, the monoclonal antibody will specifically precipitate it from NP40 extracts of these cells. This indicates that the antibody binds to a molecule which is itself included in the complex including IL-2 and the T cell receptor for IL-2 and strongly suggests that the antibody is specific for the receptor itself. The cellular proteins precipitated from the membrane of T cell blasts are found on sodium dodecylsulfate-polyacrylamide gel electrophoresis in a diffuse band between 48 and 62,000 daltons in size. The availability of monoclonal antibodies to the IL-2 receptor should greatly facilitate both the biochemical characterization of the receptor and should provide an important tool in the analysis of the means through which IL-2 acts as a growth stimulant for T cells. This has obvious importance for the utilization of agents interacting with the IL-2 receptor as immunopharmacologic agents (Malek and Shevach, LI, NIAID; Robb, Central Research Laboratories, DuPont).

Chemical characterization of B cell growth factor.

Laboratory of Immunology scientists have recently demonstrated that the proliferation of resting B cells stimulated with purified anti-immunoglobulin antibodies is, under certain circumstances, dependent upon a T cell derived factor. When highly purified mouse B cells are cultured at low cell density in the presence of low concentrations of purified anti- μ antibodies, virtually none of these cells will enter the S phase of the cell cycle. However, if

supernatants from activated EL-4 cells (T lymphoma cell line) are added to these cultures, very substantial activation occurs. The active principal within the EL-4 supernatants has been previously designated B cell growth factor (BCGF). The properties of BCGF have now been investigated. It is a material which has an approximate molecular weight of 18,000 daltons by neutral gel filtration. Isoelectric focusing reveals that it exists in two forms, one with a pI of approximately 6.4, and the second with a pI of 7.4. Digestion of the B cell growth factor with neuraminidase yields but a single species with a pI of 9.3. B cell growth factor can be separated from Interleukin-2 not only by neutral gel filtration and isoelectric focusing, but also by its behavior on phenylsepharose column chromatography. BCGF binds to phenylsepharose and is eluted from it with concentrations of approximately 10% ethandiol, substantially less than that required for the elution of Interleukin-2. On sodium dodecylsulphate-polyacrylamide gel electrophoresis, B cell growth factor migrates with a molecular weight of approximately 15,000 daltons. Finally B cell growth factor fails to bind to lines which express receptors for Interleukin-2. Thus, B cell growth factor is an entity clearly independent of Interleukin-2 which appears to be required for the progress of B cells stimulated with anti-immunoglobulin antibodies to enter the S phase of the cell cycle. Preliminary studies suggest that B cell growth factor is required relatively early in G₁ rather than at the so-called G_{1a}-G_{1b} boundary. Attempts to purify B cell growth factor by high pressure liquid chromatography are now in progress as are efforts to obtain reliable in vitro translation from mRNA as a first step in the molecular cloning of the gene which specifies B cell growth factor (Howard and Paul, LI, NIAID; Farrar, Hoffman-LaRoche, Inc., Nutley, NJ)

Activation of Resting B Cells by Non-cognate, Receptor Independent T Cell Interactions.

The analysis of the mechanisms by which resting B cells are activated has been one of the principal goals of the Laboratory of Immunology scientists. It is generally recognized that at least two distinct pathways exist for this activation process. In one, resting B cells are initially activated as a result of interaction of ligands with their receptors; antigen may interact with antigen binding receptors or anti-immunoglobulin antibodies may act as polyclonal ligands for these receptors. The second major pathway appears to involve the interaction of histocompatibility-restricted helper T cells with B cells through the recognition by the T cell of antigen and class II molecules on B cell surfaces. Such activation has been designated cognate activation. During the past year, Laboratory of Immunology scientists have identified a third major pathway of B cell activation which appears to involve the action of T cells but is neither dependent upon a receptor directed signal nor on a cognate interaction. These experiments utilized cloned, antigen specific T cell lines specific for antigens such as the terpolymer of the glutamic acid alanine and tyrosine (GAT) and highly purified populations of resting B cells. It was first demonstrated that extremely highly purified populations of resting B cells, containing at least 99% Ig cells, were very effective at stimulating T cell activation as determined by the capacity of T cells to recruit B cells to proliferate. Such highly purified B cells although they activated T cells to cause B cell recruitment, failed to cause T cell proliferation. By contrast, irradiated antigen presenting populations rich in macrophages were excellent stimulants of T cell proliferation, but

were either inferior to B cells in activating T cells for B cell recruitment or were no better than such populations of B cells. This indicates that the activation of T cells may lead to distinct consequences depending upon the nature of the antigen presenting cell involved in this activation; B cells present antigen to T cells in such a way as to favor the production of factors important in B cell recruitment, whereas, macrophages present antigen to T cells in a way that favors T cell proliferation.

A detailed analysis of the B cell recruitment that was caused by T cell activation revealed the following points: 1) Virtually all resting B cells were stimulated to enter the G₁ phase of the cell cycle by culturing them with a cloned T cell line in the presence of antigen and a major proportion (~45%) of the B cells that had entered G₁ were stimulated to enter S phase. This indicated, therefore, that B cells regardless of the specificities of their receptors could be activated by stimulated T cells. 2) There appeared to be no histocompatibility restriction in the activation of the B cells; both syngeneic and allogeneic B cells entered G₁ and S phases in response to T cell activation if a population of syngeneic antigen presenting cells, either B cells or macrophages, were present to activate the T cells. 3) By varying the concentration of antigen used for stimulation and the density of the responding cells, this non-histocompatibility restricted T cell recruitment of B cell activation could be made to appear restricted in that only syngeneic cells would be activated. This was interpreted to be due to the greater efficiency of the activation of the B cell which presents antigen to the T cell because of its proximity, than of the activation of allogeneic bystander cells. This result implies that many instances of so-called cognate, histocompatibility restricted T cell-B cell interaction may in fact represent non-cognate interactions dependent upon a T cell activating factor or set of factors capable of acting upon resting B cells without the need for receptor mediated activation (DeFranco, Ashwell, Schwartz and Paul, LI, NIAID).

Measurement of the multispecificity of the combining region of monoclonal anti-DNP antibody by affinity chromatography.

An analysis of the binding properties of antibody combining sites as well as the structure of these sites has suggested that they might express the property of multispecificity; that is, that they might bind apparently unrelated ligands through distinct portions of a relatively large combining site. A test of this concept depends upon a simple method of unambiguously measuring the affinity of interaction of a purified monoclonal antibody with a wide range of compounds, both structurally related and not structurally related to the ligand against which the antibody was raised. Laboratory of Immunology scientists have now utilized the quantitative theory of affinity chromatography of Hefcote and DeLisi and extended it so that it could be directly applied to experimental data from the binding of bivalent antibodies to haptens on affinity columns. The degree of retardation in elution time induced by given molecules compared to the elution time obtained with the index ligand can be utilized to calculate the equilibrium constant. Using a monoclonal anti-DNP antibody prepared from a hybridoma line by Dr. R. Siraganian of NIDR, a series of ligands were tested for cross-reaction with DNP. Eleven of twenty-four diverse compounds were found to bind to this monoclonal antibody with equilibrium constants between 1×10^3 liters/mole and 1×10^4 liters/mole. Of the remainder, six showed detectable binding but

equilibrium constants under 10^3 and seven showed undetectable binding. The three molecules which bound most strongly to the anti-DNP antibody were burburine chloride, monopropyl dapsone and 7-(2-hydroxyethyl) theophyllin. None of these show any obvious isostericity with DNP. This indicates that in the weak bindings observed here, one may be detecting the type of interactions anticipated from general multispecificity. This approach to the measurement of affinity makes possible an examination of bindings with equilibrium constants well below those which could be achieved under practical conditions with virtually any other technique. This approach, thus, should allow more complete examination of the antigen combining specificity of highly purified anti-DNP antibodies (Inman, LI, NIAID).

Honors, Awards and Scientific Recognition

Members of the Laboratory of Immunology play important roles in the national and international scientific community. Dr. Paul is Chairman of the Editorial Board of the Journal of Immunology and Editor of the Annual Review of Immunology. He is an advisory editor of the Journal of Experimental Medicine, a member of the editorial boards of Immunological Reviews, the Journal of Molecular and Cellular Immunology, Human Immunology and the Journal of Clinical Immunology, and an associate editor of Immunological Communications. Dr. Green is a member of the editorial boards of Clinical Immunology and Immunopathology and of Immunological Communications. Dr. Shevach is a member of the editorial boards of Cellular Immunology, the Journal of Immunological Methods, the Proceedings of the Society for Experimental Biology and Medicine and the Journal of the Reticuloendothelial Society. Dr. Schwartz is an associate editor of the Journal of Immunology and a member of the editorial board of the International Journal of Cell Cloning. Dr. Mage is a member of the editorial board of the Journal of Immunological Methods. Dr. Germain is an associate editor of the Journal of Immunology and a member of the editorial board of the Journal of the Reticuloendothelial Society.

Dr. Paul is a councillor of the American Association of Immunologists and a member of the Board of Directors of the Foundation for Advanced Education in the Sciences. He is Chairman of the U.S. Immunology Board of the U.S.-Japan Cooperative Medical Sciences Program and is a member of the Scholars Selection Committee of the Fogarty International Center, of the Committee on Promotions and Tenure, NIAID, of the Scientific Review Board of the Howard Hughes Medical Institute, of the Scientific Board of Visitors of the Oklahoma Medical Research Foundation, of the Board of Scientific Advisors of the Jane Coffin Childs Fund for Medical Research, and the Scientific Advisory Committee of the New England Regional Primate Center.

Dr. Paul received the G. Burroughs Mider Lectureship Award of the National Institutes of Health for 1982, was a plenary lecturer at the annual meeting of the American Association of Immunologists, and was an invited lecturer at a Scientific Symposium in Honor of the Two Hundreth Anniversary of Harvard Medical School, at a Symposium of the International Union of Immunological Societies in Boston, Massachusetts, at the Annual meeting of the American Society for Human Genetics, at a Symposium on the Mechanism of Lymphocyte Activation sponsored by Cornell Medical College, the Sloan-Kettering Cancer Center, and the Rockefeller University, at the III Immunological Monitoring Symposium, and at a symposium on Mechanisms of B Cell Neoplasia in Basle, Switzerland. He was a symposium chairman at the Fifth International Congress of Immunology in Kyoto, Japan.

Dr. Mage is Chairman of Division E (Immunology) of the American Society of Microbiology and President of the D.C. Chapter of Sigma Xi. She chaired a workshop at the Fifth International Congress of Immunology, and was organizer of a course, "Molecular and Cellular Mechanisms of Immunity," sponsored by the Foundation for Advanced Education in the Sciences at NIH.

Dr. Shevach was chairman of a workshop at the Fifth International Congress of Immunology, and was an invited speaker at the Gordon Conference on Immunology and Immunochemistry, at the Cold Spring Harbor Conference on

Acquired Immunodeficiency Syndrome, at the Conference on the Autologous Mixed Lymphocyte Response and at the Conference on Biology of Inflammation, Cell to Cell Interaction, Connective Tissue and Endothelium. He is a member of the Fellowship Subcommittee of the Arthritis Foundation, and of the American Association of Immunologists Program Committee.

Dr. Schwartz was a symposium speaker at the Fifth International Congress of Immunology, and an invited lecturer at the annual meeting of the British Society of Immunology, at a workshop of the U.S.-Japan Cooperative Medical Sciences Program, "Newer Technologies for Application in Immunological Research," at Hakone, Japan, and at the Gordon Conference on Immunology and Immunochemistry.

Dr. Germain was a workshop chairman at the Fifth International Congress of Immunology and an invited speaker at a workshop of the U.S.-Japan Cooperative Medical Sciences Program, "New Technologies for Application in Immunological Research," at Hakone, Japan.

Dr. Inman was an invited speaker at the Fifth International Symposium on Affinity Chromatography and Biological Recognition.

Dr. Davis was an invited speaker at the Roussel-UCLAF meeting on "Gene Organization and in Expression" in Paris, an instructor in the Cold Spring Harbor Laboratory course on "Molecular Cloning of Eucaryotic Genes," and a member of an NIAID sponsored immunology group visit to the Peoples Republic of China.

Dr. Green was elected as a member of the Austrian Society of Immunology and presented two papers to this organization in Vienna, Austria in November of 1982.

Four current or recent members of the Laboratory, Patricia Mongini, David Cohen, Maureen Howard and Thomas Malek received travel grants from the American Association of Immunologists to attend the Fifth International Congress of Immunology in Kyoto, Japan.

In addition, Laboratory members presented seminars and lectures at universities and research institutes both in the United States and abroad.

Administrative, Organization and Other Changes

Dr. David Margulies joined the Laboratory of Immunology, in a tenure track position, as a Medical Staff Fellow to establish a research program utilizing molecular genetic approaches to study lymphocyte function. His program should integrate closely with that of the other senior members of the Laboratory. Dr. Myron Waxdal left his position as Senior Investigator in the Laboratory of Immunology to accept a position in the Office of the Scientific Director, NIAID, where he will oversee the operation of a flow microfluorometry, electronic cell sorting facility. It is anticipated that in his new role he will continue to interact closely with members of the Laboratory of Immunology.

The Laboratory continues to play an important role in the training of young scientists. During the past year a series of exceptionally able young scientists completed their postdoctoral periods in the Labortory. These included David Cohen, Mark Davis, Anthony DeFranco, George Dos Reis, Ellen Heber-Katz, James Jakway, and Andrea Pavirani. Each of these individuals made very substantial contributions to the research program of the Laboratory of Immunology. In addition, Dr. Leona Fitzmaurice completed a very productive appointment as an Expert; as such she was a major element in the establishment of the Labortory's molecular biology program. A series of scientists joined the Laboratory for research training. They include Ned Braunstein, Melissa Brown, Barbara Fox, Edmundo Lamoyi, Lennart Logdberg, James McCluskey, James Miller, Hisashi Narimatsu, Junichi Ohara, Gustavo Ortega, Helen Quill, Hiroshi Suzuki, and Peter Wassmer.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00030-15 LI

PERIOD COVERED

October 1, 1982 - September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigen Recognition and Activation of Immunocompetent Cells

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

William E. Paul, M.D., Chief, LI, NIAID

COOPERATING UNITS (if any)

E. Raveche, LEP, NIADDK; J. Farrar, Hoffman LaRoche; S. Mizel, Pennsylvania State University; K. Smith, Dartmouth University

LAB/BRANCH

Laboratory of Immunology, NIAID

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.1	3.1	1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Resting B cells may be activated by anti-immunoglobulin (Ig) antibodies. This results in the entry of such cells into the G_{1a} period of the cell cycle. In the presence of high, but not low, concentrations of anti-Ig, Lyb 5^+ B cells complete G_{1a} in ~20-30 hrs and enter G_{1b} , when they are committed to entry into S phase without need for additional stimulation through receptors. Resting (G_0) B cells of both Lyb 5^+ and Lyb 5^- type can be activated to enter G_1 and S phases, without requirement for a receptor dependent stimulus, through the activity of certain activated antigen-specific T cell hybridomas and clones.

A soluble factor, B cell growth factor [BCGF], has been identified which, together with interleukin 1, allows B cells stimulated with low concentrations of anti-Ig to enter S phase. This material has a molecular weight by SDS-PAGE of ~14,000 and pI's of 6.4 and 7.4. It can be separated from interleukin 2 by phenylsepharose chromatography, tris-glycine gel electrophoresis as well as by gel filtration, SDS-PAGE, and isoelectric focusing. The further differentiation of such activated B cells into Ig secretion is based on two distinct differentiation factors - the early acting B15-TRF and the late acting EL-TRF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00035-08 LI

PERIOD COVERED

October 1, 1982 - September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Specificity in Immune Responses

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

John K. Inman, Senior Investigator, Laboratory of Immunology, 10/11N252

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology, NIAID

SECTION

INSTITUTE AND LOCATION

NIAID, Building 10, Room 11N311

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.2	0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The principal aim of this project is to test the hypothesis of general multispecificity for the combining regions of antibodies and other kinds of receptors. Receptor sites should be capable of interacting with an occasional, disparately structured substance with an affinity high enough to affect biological function. Radiolabeled antibodies or solubilized receptors are passed through small, affinity chromatography columns. Accurate measurements are made of the retention (retardation) caused by the affinity adsorbent in the presence and/or absence of many, diverse, suitably large compounds. The resulting retention values are employed directly in calculating association constants, whose occurrence frequencies provide a description of a receptor's multispecific character. Monoclonal antibodies from myelomas and hybridomas are currently being studied. In this study, large affinity probes that can be covalently bound to column matrices are being synthesized by systematic routes involving techniques used in peptide synthesis. Multispecific interactions will be usefully employed in extending the scope of specific affinity based separations and assays. Multispecificity frequencies will play a role in understanding the specificity of immune responses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00036-18 LI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ig Genetics: Ontogeny and Differentiation of Cells of the Rabbit Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Rose G. Mage, Ph.D., Senior Investigator, Laboratory of Immunology, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.5

PROFESSIONAL:

0.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies of classical genetics and of cellular expression of Ig allotypes have emphasized early events in differentiation of cells of the B lineage, attempts to develop long-term cultured B cell lines and clones, and investigations of altered phenotypic expression of allotypes in cultured cells (latent allotypes), in allotype-suppressed rabbits, and in mutant Basilea (bas) rabbits that produce Ig light chains predominantly of λ type.

The Basilea rabbits produce an unusual κ -type light chain that is present in low concentrations in sera and is found in some pre-B and B cells. We produced anti-bas allotype antisera, typed F2 and backcross progeny and found that bas segregates as an allele (or pseudallelle) of the b4, b5 and b6 allotypes. Sequenced cDNA probes from b5 allotype hybridize with mRNA species from bas rabbits distinguishable from the mRNAs encoding λ light chains. Immunoprecipitable light chains of bas κ type are distinguishable from λ chains in cell free translation products.

A subpopulation of small pre-B cells (~20%) express κ light chains and exhibit allelic exclusion. Pre-B cells expressing paternal allotype may be the source of cells with surface Ig of that type which appear in b4b5 rabbits suppressed for paternal allotype by 1 year of age and are found in proportions higher than are found in serum. In heterozygotes, VHa and κ allotypes that are imbalanced in expression in adult B cells are expressed in balanced proportions in pre-B and B cells of newborns. Cell lines from rabbit splenocytes have been maintained in culture for more than one year, are being subcloned and characterized as B cells for use in regulation, mRNA and DNA studies. We have demonstrated that cytoplasmic mRNAs produced by small numbers of cultured cells can be detected and quantitated by sensitive dot blot assays using class and allotype-specific probes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00037-16 LI
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunogenetics of Mouse Immunoglobulins and Genetic Control of Antibody Response		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Rose Lieberman, Senior Investigator, Laboratory of Immunology, NIAID		
COOPERATING UNITS (if any) M. Potter, LCB/NCI; L.A. D'Hoostelaire, Litton Bionetics, Rockville, MD.; J.M. Dorne, Washington University School of Medicine, St. Louis, MO.; G. Klein, and F. Wrener, Karolinska Institutet, Stockholm, Sweden		
LAB/BRANCH Laboratory of Immunology		
SECTION		
INSTITUTE AND LOCATION NIAID, Building 10, Room 11N322		
TOTAL MANYEARS: 3	PROFESSIONAL: 2	OTHER: 1
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>Identification of mice that have altered responses to phosphorylcholine (PC) antigens will facilitate the understanding of the <u>genetic control of immune responses</u>. Most inbred strains produce anti-PC antibodies of limited heterogeneity involving three dominant V_L-V_H pairs. In the BALB/c however, anti-PC antibodies are predominantly of the V_L-T15-V_H-K22 <u>idiotype</u> and at the DNA level have been shown to be controlled by a single gene. In other strains there are equal proportions of anti-PC antibodies of the three clonotypes. It has been shown that anti-PC response is regulated by both cellular and humoral mechanisms such as recognition of idiotypes by T_h MHC and T_h Id helper cells or by suppression of anti-Id antibody resulting in an altered anti-PC response. Recently a population of wild mice designated CNV were found which failed to make anti-PC antibodies upon immunization with various PC containing antigens. When CNV mice were mated to C.B20, an inbred responder strain, 82% of the F_1 failed to give an anti-PC responses suggesting a dominant PC gene of CNV origin. Analysis of F_2 progeny of the PC hybrids showed that mice homozygous for the CNV allotypes were PC whereas F_2 mice (CNV x C.B20) heterozygous for both allotypes and mice homozygous for C.B20 allotype expressed both PC and PC phenotypes suggesting a second <u>regulatory gene</u> that segregates independently and is not linked to allotype.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00040-09 LI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic control of immunocompetent cell interactions

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Ethan M. Shevach, M.D., Sr. Investigator, LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
3.5 2.0 1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project are to determine the role of I-region associated (Ia) antigens in the regulation of immunocompetent cell interactions and to determine the mechanism of action and site of expression of the major histocompatibility linked immune response (Ir) genes. We have analyzed the immune responses of guinea pig T lymphocytes which have been primed in vitro with allogeneic bovine insulin pulsed macrophages and have demonstrated that strain 2 macrophages were fully competent to present bovine insulin B chain to strain 13 T cells despite the fact that strain 2 guinea pigs are normally totally unresponsive to this antigen. In addition, a comparison of the reactivity profiles of self-Ia- and allo-Ia-restricted strain 13 T cells to a series of synthetic B chain peptide fragments revealed that the allo-Ia restricted populations could be activated by guinea pig insulin. These observations suggest that the clonal deletion of self-reactive cells is likely to be I-region restricted and that nonresponsiveness to any protein antigen may result from a restriction in the T cell repertoire that is generated during ontogeny by a clonal deletion mechanism of tolerance to self.

In a related series of studies, we have analyzed the fate of soluble protein antigens following pulse exposure of antigen-presenting macrophages. We have identified a critical, stable pool of antigen confined to the macrophage cell surface; no evidence was obtained to support the notion that a significant amount of antigen that was initially internalized after the pulse exposure was recompartmentalized to the cell surface.

The combined use of these genetic and biochemical approaches has resulted in a model of macrophage antigen handling that only requires surface events to yield an immunogenic moiety to be displayed in the context of Ia antigens to primed T cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00147-08 LI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Mechanism of Activation of Thymus-Derived Lymphocytes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Ronald H. Schwartz, M.D., Ph.D., Sr. Investigator, LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project attempts to understand the biochemical basis of T cell activation and the role of Ir genes in the regulation of this immune response. During the past year we have been successful at raising monoclonal antibodies specific for the antigen-specific receptor on MHC-restricted T cell clones. These antibodies inhibit antigen-induced activation of the clones and immunoprecipitate a disulfide-bonded heterodimer composed of one chain of approximately 40,000-42,000 daltons and the other of approximately 45,000-48,000 daltons. Future studies will be directed toward a further biochemical characterization of these molecules.

In a separate set of experiments we discovered that normal B cell populations can function as antigen-presenting cells provided they are unirradiated. In addition, activation of T cells for cell division and B cell recruitment were found to involve separable cellular pathways.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00148-08 LI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Lymphocyte Interactions, Receptors and Functions

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Ira Green, M.D., Sr. Investigator, Laboratory of Immunology, NIAID

COOPERATING UNITS (if any)

Vincent Hearing, Ph.D., Dermatology Branch, NCI

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
1.0 0.5 0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The L₂C leukemia is a B cell leukemia of inbred strain 2 guinea pigs. These cells have surface IgM and C3 receptors. Studies have shown that these leukemia cells possess a strong tumor specific transplantation antigen (TSTA) that can easily be demonstrated by immunization protection tests in syngeneic animals. A procedure employing KCl extraction of the leukemic cell yields a soluble extract that is also highly antigenic. The physical and chemical properties of this soluble TSTA are now the subject of study. The findings to date indicate that this TSTA has several unusual properties; it has a M.W. of 10-15,000, (as determined by Sephadex chromatography and polyacrylamide gel electrophoresis); it is resistant to boiling for 5 minutes as well as to extremes of pH. Iso-electric focusing indicates that the immunogenic material has a high isoelectric point, between 9-10. Treatment with trypsin, neuraminidase and periodate destroys the activity. These characteristics suggest that the TSTA is a basic glycoprotein of low molecular weight.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00223-02 LI

PERIOD COVERED

October 1, 1982 to September 20, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular Interactions in the Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Ronald H. Schwartz, M.D., Ph.D.

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project attempts to understand the biochemical basis for T cell interactions with other cells in the immune system. During the past year we have been able to analyze T cell activation by antigen and Ia molecules on antigen-presenting cells in terms of a receptor occupancy model, originally used to describe hormone activation of sensitive target cells. Using this model we were able to design experiments to support the hypothesis that variations in antigen dose response curves as a function of rest after antigen-induced stimulation of T cell clones are related to variations in antigen receptor number or affinity. This variation may play a critical role in the phenomenon of T cell memory.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00224-02 LI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Monoclonal antibodies as probes for T cell activation

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Ethan M. Shevach, M.D., Sr. Investigator, LI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3	1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have developed a number of murine and rat monoclonal antibodies to guinea pig and mouse T lymphocyte cell surface antigens which play critical roles in the T cell activation process. One rat monoclonal antibody (7D4) which was prepared against a murine IL-2 dependent cell line inhibited the proliferation of the immunogen to interleukin-2 (IL-2). Cell distribution studies demonstrated that the antigen defined by 7D4 was present on activated T and B lymphocytes, but was undetectable on normal murine lymphoid cells. Competition binding studies indicated that 7D4 failed to inhibit the binding of radiolabeled IL-2; however, 7D4 did precipitate radiolabeled IL-2 from detergent extracts of activated T cells that had been pulsed with IL-2. It is therefore likely that 7D4 detects an epitope on the IL-2 receptor distal to the ligand binding site or another molecule that physically associates with the receptor.

A similar approach was used to prepare a murine monoclonal antibody (5C3) to a cloned line of guinea pig alloreactive T cells. 5C3 inhibited IL-2 driven proliferation as well as antigen and mitogen induced proliferation, but did not appear to define the guinea pig IL-2 receptor because it did not inhibit the binding of IL-2 and because T cell blasts that had been stripped of the antigen defined by 5C3 were not deficient in their expression of the IL-2 receptor. However, a role for the 5C3 bearing molecule in IL-2 driven growth was suggested by the observation that the 5C3-negative blasts were unable to proliferate to IL-2.

The use of reagents such as 7D4 and 5C3 should greatly facilitate the analysis of the role of IL-2 as a universal growth hormone for T cells. These reagents are also attractive candidates for in vivo therapeutic use in attempts to selectively modulate or abrogate an ongoing immune response.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00225-02 LI
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The effects of UV irradiation on IL-1 production by keratinocytes & macrophages		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and Institute affiliation) Ira Green, M.D., Sr. Investigator, Laboratory of Immunology, NIAID		
COOPERATING UNITS (if any) Thomas Luger, M.D., Second Department of Dermatology, University of Vienna Medical School, Vienna, Austria		
LAB/BRANCH Laboratory of Immunology		
SECTION		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.75	PROFESSIONAL: 1.50	OTHER: .25
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Lymphokines are substances produced by lymphocytes and monocytes that have important functions in lymphoid cell interactions and regulation. One lymphokine, IL-1, was previously found to be produced by monocytes and macrophages. Recently, however, epidermal keratinocytes were also found to be able to produce IL-1. Since ultraviolet irradiation (UV) was found to have deleterious effects on several immune functions, both in vivo and in vitro, we investigated the effects of UV on IL-1 production by keratinocytes in vitro and in vivo and in normal and malignant macrophages in vitro. Contrary to our expectations, UV induced increased production of IL-1 in all these experiments. These results do not support the idea that the UV induced defect produced in antigen presenting cells is the result of decreased IL-1 production. Increased IL-1 production by keratinocytes after UV may also have important systemic consequences.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00226-02 LI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Rabbit Allotypes: Structure, Organization and Regulated Expression of Ig Genes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Rose G. Mage, Ph.D., Sr. Investigator, Laboratory of Immunology, NIAID

COOPERATING UNITS (if any)

E.P. Reddy, LCMB/NCI

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In order to define the organization of rabbit immunoglobulin (Ig) genes and the mechanisms that regulate their expression during lymphoid cell differentiation, we have combined our earlier approaches of immunochemistry and immunogenetics with molecular biology. Thus, we isolated rabbit Ig mRNAs of defined genetic origins, constructed and sequenced cDNA probes, and are using these probes to study expressed mRNAs and genomic DNAs prepared from genetically defined rabbits. Poly (A)⁺ RNAs were prepared from spleens of hyperimmunized rabbits of a variety of allotypes and cell free synthesis of allotype-specific γ , μ and light chains demonstrated by immunoprecipitation. Clones carrying cDNAs encoding κ light chains of b5, b9 and bas allotypes as well as heavy chain V α 1, V α 2, γ 12, 14 and μ 80 allotypes have been constructed, identified and sequenced. Some kappa chain variable region sequences from different allotypes are more similar to each other than are the sequences encoding the constant regions of b4, b5, b9 and bas "allelic" types (79-89% nucleic acid and 60-78% amino acid sequence homologies). The DNA sequences of these same molecules are remarkably homologous (93-96%) in their 3' untranslated (3'UT) regions. The high conservation of 3'UT regions enables us to detect κ light chain mRNAs from rabbits of different b allotypes on northern and dot blots. Our sequencing of DNAs encoding different rabbit Ig allotypes has allowed us to prepare and characterize allotype-specific probes. These will be used to determine the nature of mRNAs produced by purported latent-allotype producing cells cultured and manipulated in vitro, or collected from living animals. These and other studies using the probes are aimed toward achieving our goals of defining the organization and regulated expression of rabbit Ig genes in molecular terms and understanding the evolutionary origins and functional significance of complex rabbit allotypes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00227-02 LI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biochemistry of Lymphocyte Activation

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Myron J. Waxdal, Sr. Investigator, LI,NIAID

COOPERATING UNITS (If any)

Philip Noguchi

BB/DBB

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously elucidated a series of early biochemical steps in the activation pathway of murine and human T and B lymphocytes by a number of mitogenic agents. This year we studied the relevance of these pathways to the transition of activated lymphocytes from G₀ (the resting state of the cell cycle) to G_{1a} (the first state of activation).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00228-02 LI

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Membrane Associated Glyconjugates and Glycoconjugate Receptors of Lymphoid Cells

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Myron J. Waxdal, Ph.D., Sr. Investigator, LI, NIAID

COOPERATING UNITS (if any)

Claudine Kieda Centre National de Recherche Scientifique, Orleans, France
Michel Monsigny Centre National de Recherche Scientifique, Orleans, France

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	0.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lymphoid cells possess cell surface glycoconjugates which can be used as differentiation markers and also serve as receptors for mitogenic lectins. It has recently been shown that lymphoid cells also bear surface receptors for carbohydrates (endogenous lectins) similar to those found in a number of cell types from other species. These endogenous lectins are excellent candidates for active participation in cell-cell recognition, homing, intercellular signaling, and may also serve as a new set of differentiation markers.

Our studies have shown that specific endogenous lectins do exist on subpopulations of both human and murine lymphoid cells. For example, human monocytes and a subset of suppressor T cells, both bear receptors for PWCH, an arabinohydroglucan. These potent suppressor cells can be prepared by reversible agglutination with PWCH. In our present studies, we have synthesized fluorescent derivatives of PWCH for use in flow microfluorometry studies. We have also found that one of our synthetic glycoprotein probes appears to promote differentiation and immunoglobulin secretion by human B cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00229-02 LI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Lymphocyte Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Mark M. Davis, Ph.D., Staff Fellow, LI, NIAID

COOPERATING UNITS (if any)

Alfred Steinberg, A&R, NIADDK; M. Steinmetz and L. Hood, California Institute of Technology

LAB/BRANCH

Laboratory of Immunology

SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland

TOTAL MANYEARS: PROFESSIONAL: OTHER:

2.8

1.8

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to provide a molecular framework through which to analyze B and T lymphocyte differentiation and function. Initially, this has involved measuring the differences in gene expression between transformed cell lines and hybridomas which represent distinct types of B cells and T cells. These measurements demonstrate that each of these cells is very closely related and provide an important numerical basis for subsequent cloning experiments. Additionally, we developed an approach to preparing cloned cDNA libraries representing different cell type specific genes based on the use of selected cDNAs. Through this technique, we now have both B and T cell specific cDNA libraries, approximately 40 x enriched for the relevant sequences. We found that these libraries enabled the isolation of genes expressed at only a few mRNAs per cell. With this technology now in hand, we have begun to use it in the isolation of specific genes and address immunological problems. In particular we have mapped, isolated and sequenced an Ia gene (A^d), starting with genomic clones provided by Drs. M. Steinmetz and L. Hood of the California Institute of Technology. We have also identified an X-linked gene family which appears to be a maturational marker in B cells and may serve a similar function in T cells. This new gene complex may be particularly interesting in view of the numerous X-linked immunodeficiency diseases which exist. Finally, we have used this technique to analyze T cell specific cell surface receptor(s) and markers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00259-02 LI

PERIOD COVERED

October 1, 1982 - September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ia Molecules and Immune Response Genes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

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LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL MANYEARS:

0.3

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0.3

OTHER:

0

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ia molecules act as restriction (or co-recognition) elements and as immune response gene products. Thus, they are critical in antigen-recognition by T cells because 1) they are recognized by T cells together with antigen and 2) they appear to interact with and determine the immunogenicity of antigens. A series of I-A^K mutants in functional antigen-presenting cell (APC) hybridomas have been prepared. These are of three general types: Type A mutants, which appear to affect the A_g chain; Type B mutants, which appear to affect the A_g^K chain; and a set of mutants which lead to lack of expression of both A_g and A_g^K chains. The capability of these mutant APC lines to present antigen to a large panel of I-A^K restricted T cell hybridomas has been evaluated as a first step in the structure-function analysis of these mutants. At the same time, genomic clones of the A_g^K genes from mutant lines are being prepared for nucleotide sequencing so as to determine the structural basis of these mutations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00349-01 LI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Murine Class II MHC Genes and Gene Products

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

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SECTION

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OTHER:

1

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Class II (Ia) gene products play critical roles in a variety of T lymphocyte responses. They are the primary stimulating antigens in allogeneic and syngeneic mixed lymphocyte responses, they "restrict" recognition of foreign antigens by Lyt1+ T lymphocytes, and they control the ability of animals to respond to T-dependent antigens (immune response [Ir] gene function). A combination of immunological and molecular genetic approaches is being used to gain an understanding of the structural basis for this recognition of Ia molecules by T lymphocytes. Towards this goal we have cloned a cDNA corresponding to the A^d gene and utilized this probe to isolate complete λ genomic clones of A^d and A^K . These genes are being sequenced and used in DNA-mediated gene transfer experiments. Similar genomic clones corresponding to A^{α} , E^{α} , and E^{β} are being isolated to provide reagents required for producing transfected cells expressing intact two-chain Ia molecules. Mini-genomic libraries prepared from EMS derived immunoselected mutants of the TA3 (Ia $^{+}$) B cell tumor line are being constructed to permit sequence comparison of the presumed variant A^{β} or A^K genes to the wild type sequence, allowing identification of immunologically relevant regions of the Ia molecule. Regulatory sequences (promoters, enhancers) controlling the level of Ia expression will be identified by sequence and/or deletion mapping, and appropriate hybrid constructs involving inducible promoters will be made to permit us to selectively regulate the level of Ia in transfected cells. The data generated by these studies should be of significant help in enhancing our understanding of the critical structural features of Ia molecules recognized by T lymphocytes, possible sites on Ia molecules controlling interaction with antigen, and the importance of Ia density in T cell receptor occupancy and activation.

LABORATORY OF IMMUNOREGULATION
1983 Annual Report
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Summary Report
Laboratory of Immunoregulation
October 1, 1982 through September 30, 1983

Anthony S. Fauci, M.D.
Chief, Laboratory of Immunoregulation, NIAID
Deputy Clinical Director, NIAID

Studies of the Activation, Proliferation, and Differentiation of Human B Lymphocytes in Normal and Disease States

During 1981-1982, we established a model system to study the distinct phases of activation, proliferation, and differentiation of human B lymphocytes as cells are driven from the resting to the fully differentiated state. Over the past year (1982-1983), we have utilized this model system to precisely delineate the minimal and optimal signals required to drive a resting B cell through the various stages of the B cell cycle. In this system, a substantial proportion of the B cell repertoire can be activated with little or no proliferation or differentiation. Staphylococcus aureus Cowan I (SAC) has been shown to trigger human B cells to be activated in the absence of T cells and to manifest at least an initial, but self-limited, round of proliferation without terminal differentiation. Cells will not continue to proliferate unless appropriate B cell growth factors (BCGF) are added to cultures. Similar studies have been performed using anti-immunoglobulin (Ig) as the initial signal to activate normal human B cells. Data accumulated from both these systems have led to a model of B cell activation in which the initial signal activates the B cells and induces the expression of receptors for BCGF. T cell-derived BCGF when added to cultures of these activated B cells induces (in the case of anti-Ig triggering) or maintains (in the case of SAC triggering) B cell proliferation in the absence of differentiation. This capability of maintaining human B cells in a proliferative state without progression to terminal differentiation has served as the basis for studies in which B cells have been grown continuously in culture for up to eight weeks without transformation. The dissociation between proliferation and differentiation of B cells and their sequential occurrence have been demonstrated in studies in which the cells which proliferate in the presence of BCGF will not differentiate but will express receptors for a T cell replacing factor, which should more accurately be called B cell differentiation factor (BCDF) and which, in turn, induces terminal differentiation of the proliferating B cells to antibody-secreting cells.

Finally, for the first time in the human system, we have been able to identify and purify B cell subsets on the basis of their state of in vivo- or in vitro-induced activation by employing cell sizing techniques, the sequential and selective expression of cell surface activation markers defined by monoclonal antibodies developed in this laboratory, the synthesis of RNA or DNA, and the selective response to growth and differentiation factors. Thus, we have clearly defined the distinctive events which occur from the initial activation of human B cells, through proliferation, up to and including terminal differentiation and antibody production. Appreciation of these mechanisms are critical to our understanding of normal human B cell physiology as well as the aberrancies operable in diseases characterized by hypo or

hyperreactivity of B cell function (Muraguchi, Kehrl, Falkoff, Butler, Fauci, LIR/NIAID).

In addition to the polyclonal or antigen-nonspecific systems mentioned above, we have continued our studies employing the *in vitro* system of antigen-induced, antigen-specific responses of human peripheral blood B cells following *in vivo* immunization with the soluble antigens keyhole limpet hemocyanin (KLH), tetanus toxoid (TT), and pneumococcal polysaccharide (PPS). Using the KLH and TT systems, we have demonstrated that the *in vitro* induction of antigen-specific B cells which are present in the circulating B cell repertoire following primary *in vivo* immunization to secrete antibody is in fact independent of the *in vitro* binding to and direct triggering of B cells by the antigen. It is likely that the antigen-specific B cell is in a preactivated state following *in vivo* immunization and can be preferentially triggered to terminally differentiate by a nonspecific T cell factor. The specific antigen, which is introduced into culture, actually stimulates the antigen-specific T cell which, in turn, secretes such a factor. The remainder of the B cell repertoire apart from the antigen-specific B cells in question are not triggered by the T cell factor since they have not been "preactivated" *in vivo* by the immunizing antigen. In fact, under these circumstances specific antibody production is selectively triggered by T cell factor in the absence of *in vitro* antigen.

Finally, utilizing the PPS system, we have succeeded in isolating, for the first time in the human system, antigen-binding B cells which are specific for the PPS antigen. These antigen-binding cells appeared in the circulation for a well-defined and limited period following immunization. They were preactivated in that they expressed the activation antigens 4F2 and 5E9 which are defined by monoclonal antibodies developed in the LIR. These cells respond directly to growth factors similar to B cells which have already received an *in vitro* first signal, and as would be predicted, they are refractory to additional first signals such as those delivered by SAC or anti- μ . Full appreciation of the relationship between *in vivo* and *in vitro* activation of human B cells is essential to our understanding of normal B cell physiology as well as aberrancies of B cell activation in diseases characterized by B cell dysfunction (Lane, Peters, Kehrl, Falkoff, Volkman, Fauci, LIR/NIAID).

Immunoregulation of Human B Lymphocyte Responses

We had previously demonstrated in our system of antigen-induced, antigen-specific B cell responses that while concentrations of KLH in the range of 50 ng/ml induced optimal specific antibody production, a concentration of 20 μ g/ml induced specific unresponsiveness with regard to the production of anti-KLH antibody without suppression of total or polyclonal Ig production. We have now demonstrated that a complex series of events at the level of the B cell occur at these high antigen concentrations. B cells proliferate normally to these high antigen concentrations, and T cells with potent helper cell activity are induced by these concentrations. However, when antigen-reactive B cells are exposed to these high concentrations of antigen together with a differentiation signal either from a triggered T cell or a T cell factor, blockade of terminal differentiation occurs. This antigen-induced reversible induction of unresponsiveness directly at the B

cell level may be an important mechanism of modulation of B cell function in normal and disease states (Lane, Volkman, Fauci, LIR/NIAID).

We further delineated the complex modulation of this antigen-specific human B cell model by immunoregulatory T cell subsets. We examined the T cell subsets involved in antigen (KLH)-induced compared to mitogen (pokeweed mitogen [PWM])-induced help by means of limiting dilution analysis and differential radiosensitivity and demonstrated that specific and nonspecific helper T cell functions are delivered by distinct subpopulations of T cells with different precursor frequencies and different radiosensitivities. In other studies, T cells were separated into T4+ and T8+ subpopulations using monoclonal antibodies, and their modulation of antibody synthesis was studied. T4+ cells functioned as helper cells in both antigen-driven and PWM-driven cultures in a dose-dependent manner. T8+ cells suppressed the PWM-driven antigen-specific and polyclonal responses, but they did not suppress the antigen-driven responses. The reason for this lack of suppression of antigen-specific B cell responses by T8+ cells was the fact that antigen was incapable of triggering the T4+ inducers of T8+ cells, whereas PWM was a sufficient signal to trigger the T4+ inducer cells. These studies demonstrate a clear-cut dichotomy between antigen-induced and mitogen-induced human B cell responses with regard to the triggering of immunoregulatory T cell circuits (Peters, Lane, Fauci, LIR/NIAID).

For the first time in a human system, we demonstrated the role of the monocyte in the Ia-associated presentation of antigen to T cells and the genetic restriction of monocyte--T cell interactions in the induction of antigen-specific T cell-dependent B cell responses. Monocytes could present antigen to antigen-specific T cells only in the context of compatibility at the HLA-DR locus. Using cloned antigen-specific T cells (discussed below), we unambiguously demonstrated that DR compatibility between the monocyte and T cells is required at only one of the two DR haplotypes depending on the clone in question. In addition, we demonstrated the complex and multifaceted effects of monocyte-derived factor interleukin 1 (IL 1) on the regulation of human B cell reactivity. Furthermore, we demonstrated the complex positive and negative influences which allogeneic effects exert on antigen-specific B cell responses as opposed to polyclonal responses (Volkman, Gerrard, Lane, Fauci, LIR/NIAID).

Pharmacologic Modulation of Human Immune Responses

Over the past several years, this laboratory has been involved in studies aimed at delineating the precise mechanisms whereby immunosuppressive agents such as corticosteroids and cytotoxic drugs, which are used in the treatment of inflammatory and/or immune-mediated diseases, modulate the immune response in man. With regard to the effects of corticosteroids, a number of interesting observations were made including the selective suppression by chronically administered corticosteroids of human B cell function by a number of complex mechanisms including the selective enrichment in the circulation for suppressor T cells by the depletion of helper T cells. This depletion was effected by a shift in the population of helper T cells to extravascular lymphoid compartments. Additional studies were aimed at determining the mechanisms of the phenomenon, which is sometimes observed clinically whereby corticosteroids actually potentiate antibody responses under certain circumstances.

In previous years we had demonstrated that cyclophosphamide (2 mg/kg/day) administered chronically to patients with autoimmune diseases had a relatively selective effect on B cell function as opposed to T cell function. Over the past year, we have extended these studies and have made the interesting and potentially important observation that cyclophosphamide selectively suppressed distinctive phases of the B cell cycle including activation, proliferation, and differentiation to varying degrees. The proliferative responses to first signals delivered by SAC or anti- μ were profoundly suppressed. In addition, early events in the B cell activation sequence such as cell enlargement, expression of the phenotypic markers of activation (4F2 and 5E9), and the development of responsiveness to BCGF were selectively suppressed.

Finally, our most recent studies on the effects of the fungal metabolite cyclosporin A (CsA) on the various components of B cell function were most enlightening. CsA is felt to selectively affect helper T cell function. However, we have demonstrated in addition to the T cell-specific effects, rather profound, but selective, effects on certain well-defined stages of the B cell cycle. CsA had a selective inhibitory effect on the activation phase of the cell cycle versus the proliferative phase which follows the normal preactivation phase. Cell enlargement and RNA synthesis of small resting B cells triggered by anti- μ were inhibited by CsA. In contrast, once small B cells were activated by anti- μ , the resulting large, activated B cells responded normally to the proliferative signals delivered by BCGF even in the presence of CsA. Thus, CsA selectively suppresses an early step in human B cell activation and has little inhibitory effect on the subsequent factor-dependent proliferation and differentiation. CsA should prove to be a powerful tool in future studies aimed at dissecting out the various phases in the human B cell cycle (Muraguchi, Cupps, Fauci, LIR/NIAID).

Development of Human T--T Cell Hybridomas from Peripheral Blood T Cells

In 1981-1982, we developed the first panel of human T--T cell hybridomas which secreted monoclonal BCGF in the absence of T cell growth factors (TCGF) or BCDF. We subsequently described the physical and chemical properties of human BCGF. Over the past year, we have greatly extended these studies in that we have developed human T--T cell hybrids which secrete BCDF thereby allowing us to study separately the growth and differentiation phases of the B cell cycle with purified factors. We are currently performing internal labeling studies in which we will utilize these monoclonal labeled factors in experiments aimed at defining the nature of the BCGF and BCDF receptors on the target cells in question. In addition, we have developed a panel of T--T cell hybridomas which secrete either suppressor or helper factors which are currently being employed to more precisely study the immunoregulatory events in B cell function. Finally, we have fused antigen-specific T cells with our hypoxanthine/aminopterin/thymidine (HAT)-sensitive leukemic T cell partner and have developed hybrids which respond only to the antigen in question by releasing their respective growth and differentiation factors. These hybrids will be used in attempts to clone the genes coding for BCGF and BCDF (Butler, Ambrus, Muraguchi, Fauci, LIR/NIAID).

Development and Utilization of Human B Cell Hybrids from Peripheral Blood Secreting Monoclonal Antibody Against Preselected Antigenic Specificities

In 1981-1982, we developed the first human B cell hybrids from human peripheral blood which produced monoclonal antibodies against preselected antigenic specificities. We accomplished this by harvesting B cells from individuals who had been immunized with the soluble antigens KLH, TT, or PPS and fusing them with the HAT-sensitive mouse myeloma line SP-1 to produce heterohybrids which produced monoclonal human antibodies against the immunizing antigens. Over the past year (1982-1983), we have precisely delineated the conditions for optimal yield of antigen-specific hybrids.

The most critical component of the protocol was obtaining the peripheral blood lymphocytes for fusing at a time when the desired antigen-specific B cells were present in the circulation and thus readily accessible. This point cannot be overemphasized. In our studies, normal volunteers were booster immunized with TT, and their blood was drawn at various intervals ranging from 0-15 days following booster immunization. The peripheral blood lymphocytes obtained at these times were then studied for their ability to spontaneously secrete anti-TT antibodies or to form heterohybridomas secreting anti-TT antibodies. At approximately seven days following booster immunization, the number of cells in the peripheral blood spontaneously secreting anti-TT antibody as well as the ability of the cells in the peripheral blood to form heterohybridomas secreting anti-TT antibody are both at a maximum and demonstrate a kinetic pattern similar to that seen following KLH or PPS immunization. By 15 days following booster, both parameters have returned to baseline. Thus, failure to obtain the peripheral blood for fusion at an appropriate time following booster immunization will result in a drastic reduction in the yield of antigen-specific hybridomas.

Finally, we have utilized certain of our human B cell hybrids as models to dissect out several of the complex immunoregulatory mechanisms whereby T cell factors modulate B cell functional expression (Lane, Butler, Charous, Kehrl, Zhu, Fauci, LIR/NIAID).

Cloning of Antigen-Specific T Cells from Human Peripheral Blood

From 1980 through 1982, we succeeded in developing the first panel of human T cell clones from peripheral blood which were reactive with soluble antigens to which the host had been immunized. We demonstrated that these were true clones, were antigen specific, and required antigen-presenting cells (APC) which were major histocompatibility complex (MHC) compatible at one of the two DR loci. Over the past year, we have extended those studies and have developed a panel of antigen-specific clones from the same individual and have demonstrated that an individual clone could recognize antigen in the context of APC bearing only one of the two DR haplotypes of the donor of the clone, but not APC bearing the other of the two DR haplotypes. On the other hand, another individual clone from the same donor could recognize antigen in the context of APC bearing the DR haplotype which was not recognized by the first clone and could not recognize antigen in the context of APC with the DR haplotype which the first clone did recognize. Thus, we have demonstrated that in human as in other species individual antigen-specific T cells are MHC restricted to a single self molecule encoded in the human Ia region which may be derived from either parental Ia gene. In addition, Epstein-Barr virus (EBV)-transformed B cells were shown to also present antigen in an MHC-restricted manner. Also, T cell clones were established which bore the T4 helper/inducer phenotype and which provided help to B cells in an

MHC-restricted and antigen-specific manner. Finally, T cell clones were established which secreted a variety of factors such as IL 2, BCGF, BCDF, and gamma interferon. Availability of T cell clones in the human system should prove to be extremely helpful in the delineation of the fine specificity of T cell responses as well as in endeavors towards defining the nature of the T cell receptor for antigen (Volkman, Margolick, Fauci, LIR/NIAID; Matis, Medicine Branch/NCI).

Studies in the Acquired Immune Deficiency Syndrome (AIDS)

Over the past year, the LIR has been intensely involved in the study of AIDS. Our work has centered on four main areas: description of the clinical, pathologic, and pathophysiologic manifestations of AIDS; characterization of the immunologic dysfunction in AIDS; searching for a causative agent(s); and development of therapeutic strategies.

We have demonstrated that the common denominator of the immunopathophysiology of AIDS is a profound immunosuppression due to a selective qualitative and quantitative depletion of the helper/inducer subset of T lymphocytes. This immunologic lesion results in a degree of profound acquired immune deficiency, which rivals the most potent chemotherapeutic agents. In combination with as yet ill-defined environmental or genetic factors, this immunologic defect results in the development of frequent, varied, and often fatal opportunistic infections or unusual neoplasms. We have found that the degree of immunologic decline plays a major role in the clinical diversity which patients with this syndrome exhibit.

Studies of the nature of the immunologic defects in AIDS have yielded many interesting findings. While the helper/inducer subpopulation of T lymphocytes is severely compromised, the cytotoxic/suppressor population appears capable of functioning normally when provided with the appropriate signals such as gamma interferon, IL 2, or normal allogeneic T4 lymphocytes. These *in vitro* observations have been our guide in the development of therapeutic protocols. In addition to these abnormalities of T cell function, patients with AIDS have been characterized as having profound abnormalities of B lymphocyte activation and immunoregulation in the form of polyclonal B cell activation, a relative lack of resting B lymphocytes, and an inability to mount a serologic response to a *de novo* protein antigen. These findings suggest a viral transformation of B lymphocytes in the absence of the normal regulatory T cell influences and cast doubt upon the reliability of serologic diagnosis in patients with AIDS as well as providing insight into the explanation for the autoimmune phenomena seen in this and related patient groups.

The search for an etiologic agent(s) has thus far been unsuccessful. Although numerous unusual agents have been isolated from patients with AIDS, none seem to fulfill the criteria needed to be implicated as anything other than an unusual opportunist. If the agent(s) are trophic for the T4 lymphocytes, then studies on whole blood or unfractionated lymphocytes may not contain a sufficient proportion of infected cells to make a diagnosis. Accordingly, our studies are currently being carried out in collaboration with other laboratories to study isolated, purified populations of T4 lymphocytes, either as fresh isolates from peripheral blood or in the form of T cell lines and clones.

We have been intensively involved in studies aimed at establishing the feasibility of reconstituting immune function in these patients, either through the use of cellular transfers or soluble mediators of the immune response. We have performed a partial immune reconstitution in a patient with AIDS through the use of peripheral lymphocyte transfer and bone marrow transplantation from his healthy identical twin. Despite the fact that partial immunity was transiently restored and adaptive transfer of immunity to the soluble protein antigen KLH was accomplished, the patient has continued to develop opportunistic infections and progressive Kaposi's sarcoma. This study has been of landmark importance since it clearly demonstrated that simple lymphocyte replacement by either of these two techniques alone is probably inadequate in AIDS, presumably due to a persistence of the causative infectious agent in the host. Finally, clinical trials are underway utilizing the T cell-derived lymphokines gamma interferon and IL 2. Studies in AIDS are of high priority not only because of the seriousness of the syndrome with regard to its real and potential public health hazard but also because it serves as an extraordinary model for this laboratory to intensively study a disorder of immunoregulation with all its implications for host defenses against infectious diseases and neoplastic transformation (Lane, Fauci, LIR/NIAID; Masur, CCM/CC).

Mechanisms of Abnormal Cellular Activation and Immunoregulation in Immune-Mediated Diseases

Over the past year, we have studied the abnormalities of B cell triggering and immunoregulation in certain autoimmune diseases, particularly Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE). In SS, we demonstrated a localized hyperreactivity of B cells in the accessory salivary glands without the severe B cell and T cell abnormalities seen in patients with SLE. In addition, we have recently demonstrated a high incidence of free monoclonal lambda light chains in the sera of patients with SS, which adds credence to the hypothesis that this disease begins as a monoclonal proliferation of B lymphocytes.

We further extended our previously reported studies on the B cell abnormalities in SLE. This year, we have demonstrated that B cells in patients with SLE are pretriggered *in vivo* to the extent that they are able to directly respond to the nonspecific signals provided by T cell-derived BCGF and BCDF. Thus, they are much more susceptible to B cell hyperreactivity in response to a whole host of nonspecific signals. This may well explain the global hyperreactivity at the B cell level, which is so characteristic of SLE (Lane, Falkoff, Fauci, LIR/NIAID).

Delineation of the Selective Defect in Natural Killer (NK) Cell Activity in the Chediak-Higashi Syndrome (CHS) and Its In Vitro Pharmacologic Correction

We have extended our previously reported observation of a selective defect in NK cell activity in patients with CHS. This was the first description in man of a primary immunodeficiency disease with an isolated defect in NK cell activity. This has potential clinical implications since CHS patients have a propensity to develop lymphoid malignancies. Over the past year, we have demonstrated that the defect was at the level of the "active" NK cell in that patients with CHS have normal numbers of NK cells and normal percentages of target-binding NK cells. The defect was demonstrated to

be a functional defect in that NK cells bind to target cells. We then demonstrated by in vitro studies that cyclic guanosine monophosphate (cGMP) or inducers of cGMP could correct the defect in NK function in CHS. These latter studies underscore the role of cyclic nucleotides in the regulation of NK function and raises the possibility of a therapeutic modality in patients with defects in NK function (Katz, University of Florida; Fauci, LIR/NIAID).

Studies in the Idiopathic Hypereosinophilic Syndrome (HES)

Over the past year, we have continued and extended our clinical and pathophysiologic studies in a large number of patients with HES. The precise mechanisms of organ system damage and dysfunction were delineated predominantly with regard to the endomyocardial fibrosis of HES. The major basic protein of the eosinophil was demonstrated to be directly toxic to endothelial cells and likely accounts for the first insult to the endocardium. This was followed by a laying down of platelet thrombi with subsequent mural thrombosis and endocardial fibrosis leading to the restrictive cardiomyopathy. Although a portion of these studies were performed in an in vitro system, they demonstrate the potential role of an eosinophil product in the direct damage to human tissue in HES. With regard to clinical studies, aggressive medical and surgical approaches to the heart disease as well as chemotherapeutic regimens aimed at the underlying eosinophilia have led to a dramatic improvement in the prognosis of HES. These patients continue to serve as a valuable model to study the diverse and complex functional properties of human eosinophils (Fauci, LIR/NIAID; Gralnick, Hematology Service, CC; Gleich, Mayo Clinic).

Clinical, Immunologic, Pathogenic, and Therapeutic Studies in the Spectrum of Vasculitis

Over the past 15 years, we have been prospectively studying the largest group of patients with the vasculitic syndromes of any center in the world. Over the past year, these studies have continued and accelerated. Clinical, pathophysiologic, immunopathogenic, and therapeutic results have allowed us to design a revised categorization scheme for the vasculitides which has reached worldwide acceptance. Fifteen years ago, we designed and implemented an aggressive chemotherapeutic regimen consisting of chronic low-dose cyclophosphamide together with alternate-day corticosteroids for treatment of several of these formerly universally fatal diseases such as Wegener's granulomatosis and systemic vasculitis of the polyarteritis nodosa group. Using this regimen, we have affected long-term remissions and cures in greater than 90% of patients. This landmark study has now led to this regimen being successfully used throughout the world and has completely changed the previously grave prognosis of these diseases to one of almost invariable remission. We have extended these studies and now are treating a number of other vasculitic syndromes such as isolated central nervous system vasculitis, Takayasu's arteritis, and the systemic vasculitis of the connective tissue diseases with similar success.

Furthermore, these patient populations have been utilized to precisely delineate aberrations of activation and immunoregulation of lymphoid cell function in man and have served as excellent models for the concomitant study of the normal human immune system. In addition, the precise effects of various therapeutic regimens, particularly corticosteroids and cytotoxic

agents, on human lymphoid cell activation and immunoregulation have been described (Fauci, Cupps, Volkman, Lane, LIR/NIAID).

Future Plans and Objectives

Future plans and directions for the project on human lymphocyte activation and immunoregulation include studies aimed at more precisely delineating the model which we have proposed for the activation, proliferation, differentiation, and immunoregulation of human B lymphocyte function. We have thus far successfully defined the initial steps of activation dissociated from proliferation of B cells. In this system, triggering of B cells by minimal concentrations of anti- μ antibody will drive the cell from a resting state to an activated state at which point it expresses receptors for B cell growth factor (BCGF). We have demonstrated that the activated B cell will then continuously proliferate if supplied with T cell-derived BCGF. We have employed a monoclonal BCGF produced in our laboratory from a human T-T cell hybrid. Although we have demonstrated that an activated and proliferating cell will differentiate if exposed to a differentiation factor, purified B cell differentiation factor is not yet available. Studies are underway aimed at this goal. We feel confident that we will be able to continue to develop and employ purified immunoregulatory factors, which will then allow us to dissect out the cascade of events in human B cell activation. We are currently internally labeling these factors using the hybrid lines which we have developed. We will use these labeled monoclonal factors to study their cellular receptors and the sequential appearance and disappearance of these receptors during various stages of B cell activation in normal and disease states.

We will place a major effort in the continued study of the mechanisms of response of in vivo-activated B cells to various differentiative and tolerogenic signals. We have described a model system whereby preactivated (by in vivo immunization) B cells respond to antigen-specific and nonspecific T cell factors in a non-major histocompatibility complex (MHC)-restricted, antigen-independent manner despite the fact that the monocyte-T cell interaction which triggers the release of factor is strictly MHC restricted and quite antigen specific. In this regard, we will pursue the delineation of the functional capacities and restrictions of various phenotypically and functionally distinct subsets of human B cells as well as the various subsets of immunoregulatory T cells.

Now that we have developed our current model of human B cell activation, proliferation, differentiation, and immunoregulation, we will study the precise effects of corticosteroids and cyclophosphamide on each of the steps of this cascade. Since most of the diseases which we are currently studying at the LIR are treated with either or both of these agents, such studies should prove to be extremely informative and fruitful.

The three major components of the cloning project have been: 1) the development of human B cell hybrids from human peripheral blood which secrete monoclonal antibody against antigens of predetermined specificity following in vivo immunization; 2) the development of T-T cell hybrids from human peripheral blood T cells; these hybrids have been shown to secrete a variety of immunoregulatory factors; and 3) the development of antigen-specific human T cell clones which are MHC restricted and which express immunoregulatory functional capabilities.

Studies over the coming year will be directed at pursuing and continuing each of these fruitful projects. Further delineation of the precise conditions for optimal development of human B cell hybrids is under way. We will be attempting to fuse hepatitis B-specific B cells in order to produce large quantities of hepatitis B-specific antibody. In addition, the hybrids will be used as a model to study B cell function in a monoclonal system.

We will continue to develop T--T cell hybrids and will employ the technical extension of fusing T cell clones with our parent T cell leukemia cell line. In this manner, we can fuse cells whose function has been already precisely defined by subcloning and functional study. We will also be developing monoclonal antibodies against these monoclonal factors produced by our T cell hybrids. This will allow us to develop sensitive assays for the detection of such factors.

We will continue our studies on T cell clones, particularly with regard to further defining the fine specificities of antigen recognition as well as MHC restrictions. We will also be initiating studies in which we will clone T cells isolated from tissues of our patient populations with various immune-mediated diseases as well as diseases such as lymphomatoid granulomatosis, which we feel is a T cell-derived premalignant state.

Finally, a molecular biologist will be joining the LIR this year, and we will initiate a series of studies directed at cloning the genes of our various hybridized lines and clones which express definable functional capabilities. In addition, we will study the gene rearrangements associated with the process of B cell activation.

Studies on the immunopathogenic mechanisms of certain immune-mediated diseases will continue. Patients with a variety of diseases characterized by aberrancies of immune function will continue to serve as a source of material for studies aimed at delineating the mechanisms of immune dysfunction. Since we have now established our model of B cell activation, we will direct studies at more precisely defining the level of defect or abnormality of B cell activation and/or immunoregulation in certain of these diseases by integrating our studies into the framework of the discrete steps of B cell activation, proliferation, differentiation, and immunoregulation. In addition, we will utilize our newly developed cloning and hybridizing technology in the study of subpopulations of lymphoid cells obtained from the peripheral blood, bone marrow, and other lymphoid tissues as well as nonlymphoid organs of the patients. This will allow us to add an unprecedented degree of precision to the study of cellular subsets involved in the pathogenesis and expression of certain of these diseases.

Of particular note are our plans for the study of the acquired immune deficiency syndrome (AIDS). In addition to performing the same types of studies described above in patients with AIDS, we will be engaging in a series of studies aimed at determining the feasibility of immunologic reconstitution of these patients. In this regard, we have recently performed an adoptive transfer of peripheral blood lymphoid cells from a well heterosexual male into his homosexual AIDS identical twin and have successfully transferred reactivity to a number of carefully controlled immunizing antigens such as keyhole limpet hemocyanin. The reconstitution was successful, not total, and temporary (lasting two to three months). We have then gone on to perform a

bone marrow transplant from the well to the AIDS identical twin. Again, only partial and temporary reconstitution was accomplished suggesting the continued presence of the putative infectious immunosuppressive agent in the host.

Over the coming year, we will continue our studies with therapeutic trials of lymphokines such as gamma interferon and IL 2 in an attempt to at last partially reconstitute the deficient immune response of AIDS patients. Finally, based on our studies in which the immune defect at the T cell level was shown to be a selective quantitative and qualitative abnormality of T4 cells, we will carefully examine cloned populations of AIDS T4 cells for the presence of viral infection by lymphocytotropic T cell viruses.

Administrative, Organization, and Other Changes

The Laboratory of Immunoregulation (LIR) was established in 1980 and is now three years old. The theme of the LIR is to study the precise mechanisms of activation and immunoregulation of human immunocompetent cells, particularly B lymphocytes, in normal individuals and in a variety of disease states characterized by abnormalities of immune function. In addition, the LIR continues to conduct the bulk of clinical studies carried out in the NIAID intramural program within the Clinical Center.

When the LIR was established, the plan was for gradual expansion with the development of individuals predominantly from the existing Clinical Associate and Medical Staff Fellow pool, who would remain within the program for variable periods of time both to maintain the continuity in ongoing studies as well as to serve as a source of individuals who might potentially and ultimately assume senior positions in the laboratory. In addition, when appropriate and necessary, individuals would be brought in from the outside as the program expands. In this regard, Drs. H. Clifford Lane and David J. Volkman have been designated as Senior Investigators, who will be remaining in the program. They were formerly Clinical Associates, who had remained in the program for an additional one to two years. Similarly, Dr. John H. Kehrl, who will complete his three-year Clinical Associateship in June 1983, will be remaining within the Public Health Service for an additional one to two years with the possibility of establishing him as a Senior Investigator in the future. Dr. Atsushi Muraguchi, who came to the laboratory in 1981 as a Visiting Fellow from Osaka, Japan, will be remaining on for at least another year. Drs. Lauren Charous, Joseph Margolick, and Randi Leavitt are completing their second year as Medical Staff Fellows and will begin their third year in July 1983. It is most likely that one or two of these individuals will be remaining on for an additional year after their third year, as indicated above. Drs. Julian L. Ambrus and Debra L. Bowen will enter their second year as Medical Staff Fellows in July 1983, and Dr. Harris Goldstein will join us in July 1983 as a first-year Fellow to begin his clinical year on the NIAID Clinical Service. Dr. Theresa L. Gerrard will remain on an additional year as a Staff Fellow. In November 1983, Dr. Le Thi Bich-Thuy from Prof. Jean-Pierre Revillard's group at the Hopital Herriot in Lyons, France, will join the LIR as a Visiting Fellow. Finally, in the fall of 1983, Dr. Ulrich Siebenlist from Dr. Philip Leder's laboratory at Harvard Medical School will join the LIR first as a Visiting Associate to be later converted to a Senior Investigator. He will provide the LIR with expertise in molecular biology. We will be hiring an additional technician to assist him.

Leaving the LIR this year will be Drs. Thomas R. Cupps, Reuben J.M. Falkoff, and Joseph L. Butler, who have completed five, four, and three years respectively as Clinical Associates; Dr. Marion Peters, who has completed two years as a Visiting Fellow; and Dr. Li-Ping Zhu, who has completed two years as a Guest Worker from the People's Republic of China.

Ms. Ann C. London has completed her first year as Editorial Assistant to the LIR and has proved to be invaluable to our efforts. Ms. Mardell Wilson has left the LIR as Laboratory Chief's secretary. Mrs. Sarahlea Kemp has replaced Mrs. Paula C. Kendros as Clerk (Typist).

The laboratory space has been consolidated such that now the entire laboratory is located on the B Wing of the 11th floor in the Clinical Center. Module 11B-05 is being renovated to provide space for Dr. Siebenlist upon his arrival in October 1983.

Honors, Awards, and Scientific Recognition

Over the past year, members of the Laboratory of Immunoregulation, predominantly in the person of Dr. Fauci, Chief, LIR, have received a number of awards and honors. First, Dr. Fauci has served on a number of committees of scientific note. He is currently the Program Chairman for the national meeting of the American Association of Immunologists. He is serving on the Committee on Clinical Immunology and Immunopathology of the American Association of Immunologists. Dr. Fauci is also a representative of The American Association of Immunologists to the Examinations Committee of the American Board of Allergy and Immunology. In addition, Dr. Fauci is on the postgraduate education committee of the American Academy of Allergy and is a member of the prestigious Section of Physiology in Clinical Science of the American Physiology Society. It is of note that he is the only immunologist on this well-known section on Physiology in Clinical Sciences which is organized and run by the American Physiology Society. In addition, he is a member of the subcommittee on the classification of vasculitis of the American Rheumatism Association and is on the ad hoc committee of the National Foundation for Infectious Diseases. He has been appointed to the American Federation for Clinical Research President's Public Policy Advisory Committee. Finally, he was elected this year to the Board of Directors of the American Board of Allergy and Immunology.

Dr. Fauci serves on a number of editorial boards of journals concerned with the areas of immunology, allergy, and infectious diseases. He is the Section Head of the Editorial Board of the Clinical Immunology Section of The Journal of Immunology. In addition, he is the Associate Editor in charge of Allergy and Immunology of the American Journal of Medicine. Dr. Fauci also serves on the editorial boards of The Journal of Clinical Investigation, The Journal of Infectious Diseases, The Annals of Allergy, The Journal of Immunopharmacology, The Journal of Clinical Immunology, the new series entitled Clinics in Immunology and Allergy, the new journal EOS, Clinical and Experimental Rheumatology, the Italian journal La Ricerca, Clinical Immunology and Immunopathology, and the Physicians' Journal Update. In addition, Dr. Fauci in collaboration with Dr. John I. Gallin of the LCI/NIAID, has created and is the co-editor of the newly established series ADVANCES IN HOST DEFENSE MECHANISMS. Finally, together with Dr. Lawrence Lichtenstein, Dr. Fauci is editing a new textbook entitled CURRENT THERAPY IN ALLERGY AND IMMUNOLOGY. Dr. Fauci has also contributed invited chapters on a number of subjects for most of the major textbooks of medicine as well as subspecialty textbooks in immunology, allergy, and infectious diseases.

As part of the recognition for scientific accomplishments, clinical investigators may be asked to visit outside institutions and serve for periods of from two to three days as visiting professor within a given institution. In this regard, Dr. Fauci had been asked and did serve as visiting professor at several major institutions during the year. Among these were his visiting professorships at the University of Colorado in Denver and Tufts-New England Medical Center in Boston.

In addition, Dr. Fauci was asked to give several major or named lectureships during the year. He was the Scientific Workshop Chairman and Plenary Lecturer at the VIII Pan American Congress of Rheumatology in Washington, D.C. He was an invited Plenary Speaker at the 14th Symposium of

the Collegium Internationale Allergologicum in Sorrento, Italy. He was an invited Plenary Lecturer at the American Society of Hematology National Meeting. He delivered the State of the Art Lecture to the American Society of Nephrology National Meeting. He was the Chairman of a Scientific Workshop of the 15th International Leucocyte Culture Conference at Asilomar, California. He delivered the prestigious Thomas Dent Mutter Lecture to the College of Physicians of Philadelphia on the "Acquired Immune Deficiency Syndrome." He was the Visiting Lecturer of the Fellows of the Scripps Clinic and Research Foundation, and he was an invited Symposium Speaker at the Annual Meeting of the American Association of Immunologists. Finally, the work of the LIR and its members were recognized in that ten papers from the laboratory were chosen to be presented at the highly competitive program of the National Meeting of the AAP/ASCI/AFCR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00210-03 LIR

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunoregulation of Human Lymphocyte Function in Normal and Disease States

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Anthony S. Fauci, M.D., Chief, LIR/NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.5	3	1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A model of human B cell activation, proliferation, and terminal differentiation was established. Human B cell subsets were identified and separated on the basis of their state of activation by employing cell sizing techniques, the sequential and selective expression of cell surface markers, the synthesis of RNA or DNA, and the selective response to growth and differentiation factors. The selective sensitivity of various phases of the human B cell cycle to pharmacologic modulation by agents such as cyclosporin A, corticosteroids, and cytotoxic agents was demonstrated. In an antigen-specific system of human B cell activation, immunoregulatory T cell and monocyte subsets were identified, and the diverse mechanisms of their modulation of B cell responses were delineated. Radiation sensitivity and precursor frequency studies of immunoregulatory T cells as well as the limiting dilution of analyses of antigen-specific B cells were performed. The phenomenon of high antigen concentration blockade of B cell differentiation in the face of normal B and T cell proliferation was described. The relationship between the primary activation of B cells by antigen in vivo and the subsequent antigen-dependent driving of these cells towards terminal differentiation in vitro by nonspecific, non-major histocompatibility complex-restricted soluble T cell factors was studied. For the first time in the human system, antigen-binding B cells were identified and purified from the peripheral blood following in vivo immunization with soluble antigens such as pneumococcal polysaccharide. The positive and negative influences of allogeneic effects on antigen-specific human B cell responses were studied, and the T cell subsets responsible for these effects were described. The genetic restriction of monocyte--T cell interactions in the induction of T cell-dependent B cell responses as well as the role of Ia and certain cell surface activation molecules in the antigen-induced activation of T cell-dependent B cell responses were described.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00211-03 LIR

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of Human Lymphocyte Subsets Employing Cloning and Hybridoma Technology

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Anthony S. Fauci, Chief, LIR/NIAID

COOPERATING UNITS (if any)

None

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SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4	3	1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Substantial advances were made in the development of human T--T hybridomas which secrete a variety of immunoregulatory factors. These hybrids were established by the fusion of human blood T cells and a hypoxanthine/aminopterin/thymidine (HAT)-sensitive human T cell leukemia line. We developed the first human T--T cell hybridoma which secreted monoclonal B cell growth factors (BCGF) devoid of T cell growth factor (TCGF) or B cell differentiation factor (BCDF) thus providing a source of purified factor for the continuous growth of human B cell lines as well as a source of factor to dissect out the distinct phases of the B cell cycle of activation, proliferation, and differentiation. In this regard, we most recently developed hybrids which elaborated BCDF devoid of TCGF activity. These cloned hybrids lines were used to precisely characterize the nature of the growth factors and to initiate studies aimed at defining the nature of the growth factor receptors on the target cells in question. Such studies were previously not feasible with the crude factor-containing supernatants derived from cultures of heterogenous mononuclear cells. Model systems of human B cell hybridomas which had originally been developed in this laboratory were perfected with regard to the delineation of the precise conditions for optimizing the yield of antigen-specific B cell hybrids from the peripheral blood of immunized subjects. Improved methodologies for the cloning of antigen-specific human T cells from peripheral blood were developed and resulted in the production of T cell clones which were antigen specific, were MHC restricted with regard to DR or MB antigen-bearing presenting cells, and expressed functional helper activity. Furthermore, certain of these antigen-specific T cell clones were shown to secrete a variety of immunoregulatory T cell factors such as interleukin 2, gamma interferon, BCGF and BCDF.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00212-03 LIR

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of the Immunopathogenic Features of Immune-Mediated Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Anthony S. Fauci, M.D., Chief, LIR/NIAID

COOPERATING UNITS (if any)

None

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Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 4 PROFESSIONAL: 2.5 OTHER: 1.5

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies on the immunopathogenesis of a diversity of immune-mediated diseases and/or diseases characterized by aberrancies in immune function were performed. An intensive effort was directed at the acquired immune deficiency syndrome (AIDS). We precisely delineated the nature of the immune defect in AIDS and demonstrated that patients manifested a profound selective quantitative and qualitative defect in the T4 (Leu 3) inducer/helper subset of T lymphocytes. This has important implications in localizing the target cell of the putative infectious agent of AIDS to the T4 lymphocyte. In addition, we demonstrated a significant abnormality in B cell function in AIDS characterized by a polyclonal hyperactivity indicative of an in vivo triggering of multiple clones of B lymphocytes. We carried out a series of studies aimed at reconstitution of the defective immune function of AIDS patients. In this regard, we performed a series of lymphocyte transfers as well as a bone marrow transplant from a heterosexual well male to his homosexual identical twin brother with AIDS. Only partial reconstitution of immune function was effected and the patient continues to deteriorate suggesting that the putative agent of immunosuppression may still persist in the patient. We have also carried out studies of infusions of gamma interferon into AIDS patients in the hope of effecting at least partial reconstitution. Only equivocal results were seen. Our previous studies in which we described the first example of a primary immunodeficiency disease with an isolated defect in natural killer (NK) cells in patients with the Chediak-Higashi syndrome were extended this year. We demonstrated that patients with this syndrome have normal numbers of NK cells but have a functional defect in "active" NK cells. Finally, mechanisms of aberrant B cell activation and immunoregulation were delineated in several autoimmune diseases such as systemic lupus erythematosus and Sjogren's syndrome.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00213-03 LIR

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical, Immunopathogenic, and Therapeutic Studies in the Spectrum of Vasculitis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Anthony S. Fauci, M.D., Chief, LIR/NIAID

COOPERATING UNITS (if any)

None

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Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION

NIAID NIH Bethesda, MD 20205

TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0
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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The LIR is currently prospectively studying the largest group of patients with the vasculitic syndromes in the world. On the basis of clinical, pathophysiological, immunopathogenic and therapeutic results obtained over the past 15 years, we have designed a revised categorization scheme for the vasculitides which has now reached worldwide acceptance. We have developed and instituted aggressive chemotherapeutic regimens consisting of chronically administered cyclophosphamide together with alternate-day corticosteroids in several, formerly universally fatal diseases such as Wegener's granulomatosis. In this regard, over the past year we reported our long-term follow-up of 85 patients with Wegener's granulomatosis in which we demonstrated a 93% remission and cure rate. We have now applied these approaches with remarkable success to other of the vasculitic syndromes such as systemic vasculitis of the polyarteritis nodosa group, isolated central nervous system vasculitis, Takayasu's arteritis, and the acute vasculitis of Sjogren's syndrome. In addition, we studied the pathophysiology of lymphomatoid granulomatosis and have shown it to be a pre-lymphomatous condition which responds in its early stages to chemotherapeutic regimens used in the vasculitic syndromes. Patients who responded in the early stage did not go on to lymphoma and remained disease free over long-term follow-up. The patient populations studied in the vasculitis protocol have been utilized to precisely delineate aberrancies of lymphocyte activation and immunoregulation seen in these diseases. In addition, the precise effects of various therapeutic regimens, particularly corticosteroids and cytotoxic agents, on human lymphoid cells have been described. In this regard, we demonstrated the exquisite and selective sensitivity of certain phases of the B cell cycle to cyclophosphamide therapy, an observation which might help explain its efficacy in certain diseases characterized by hyperreactivity of B cell function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00381-01 LIR

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Therapeutic Modalities in the Acquired Immune Deficiency Syndrome (AIDS)

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)
Anthony S. Fauci, M.D., Chief, LIR/NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A variety of therapeutic modalities were attempted in AIDS patients. In an attempt to reconstitute the defective immune function of a single patient, we performed a bone marrow transplantation from a well heterosexual male to his homosexual twin brother with AIDS. In addition, we periodically transfused peripheral blood lymphocytes from the well to the AIDS twin. Partial reconstitution of immune function was achieved with transfer of delayed cutaneous hypersensitivity responses to the soluble antigen keyhole limpet hemocyanin (KLH), quantitative increase in the OKT4 subset of T cells, and an increase in blastogenic and cytotoxic lymphocyte function. Despite this, the reconstitution was only partial and had little impact on the clinical status of the patient. However, the study demonstrated the feasibility of using this approach as one of the therapeutic modalities in AIDS. A therapeutic trial of the administration of immune (gamma) interferon to AIDS patients was undertaken. This resulted in mild to modest improvement in the lesions of Kaposi's sarcoma and transient increases in certain in vitro immunological parameters. However, these changes were not impressive, nor were they of lasting duration. In general, patients continued to deteriorate clinically despite the modest improvements that were sometimes seen. Based on the demonstration in vitro that interleukin 2 (IL2) markedly enhanced and normalized the defective natural killer cell activity as well as the cell-mediated cytotoxicity against cytomegalovirus-infected targets of lymphocytes from AIDS patients, we are undertaking a trial of the administration of IL2 to patients with AIDS. In addition, we are currently examining the effects of repeated plasma exchange in AIDS patients. This study is based on the findings from other laboratories that AIDS patients have circulating inhibitors of immune function which may be compounding the underlying immunological defect. Future studies will be directed at combining several of the above-mentioned treatment modalities in an attempt to maximize our ability to reconstitute the defective immune function in AIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00382-01 LIR

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
 The Inheritance of the Immunological Defects in
 AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)
 Anthony S. Fauci, M.D., Chief, LIR/NIAID

COOPERATING UNITS (if any)
 None

LAB/BRANCH
 Laboratory of Immunoregulation

SECTION

INSTITUTE AND LOCATION
 NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.5	OTHER: 0.5
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CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies were performed which precisely delineated the immunological defects in AIDS. Patients manifest a total lymphocytopenia with a selective quantitative deficiency in the helper/inducer subset of T lymphocytes phenotypically defined by OKT4 or Leu 3 monoclonal antibodies. Of particular note was the finding that the OKT4 subset of cells was also found to be qualitatively defective in that they manifested a profound deficiency in inducer/helper function. Although the OKT8 subset of T cells from AIDS patients functioned normally as suppressor cells when provided with a source of normal T4 cells, they were in an abnormally activated state expressing the Ia and T10 antigens characteristic of activated T cells. The selective quantitative and qualitative defect in OKT4 cells is compatible with one of the potential etiologies of AIDS which is an infection of OKT4 cells with a lymphocytotropic virus such as a retrovirus. AIDS patients also manifest a defect in natural killer (NK) cell activity as well as cell-mediated cytotoxicity against cytomegalovirus-infected target cells. Of particular note was the finding that in vitro incubation of AIDS lymphocytes with interleukin 2 (IL2) resulted in a dramatic normalization of the NK cell as well as the cytotoxic responses against viral infected target cells. This served as the basis for the institution of a trial of in vivo IL2 administration to AIDS patients in an attempt to at least partially reconstitute their defective immune function. AIDS patients were also shown to be defective in their cell-mediated and humoral immune response to in vivo immunization with soluble antigens such as keyhole limpet hemocyanin (KLH). Furthermore, their lymphocytes did not respond in vitro to KLH stimulation with either a blastogenic or antibody response. Finally, we demonstrated a profound defect of B cell function in AIDS patients. Their B cells are profoundly activated in vivo such that they are virtually all in the activated state, strongly suggesting that they are responding in an abnormal fashion to an antigenic (probably viral) stimulus in the absence of adequate T cell control. Secondary to this abnormal in vivo B cell activation is a defect in the ability of these cells to be triggered in vitro by an activation signal.

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SUMMARY STATEMENT

Annual Report

Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
October 1, 1982 to September 30, 1983

HEPATITIS

Hepatitis A Virus (HAV). HAV is responsible for most epidemics of short incubation hepatitis and thus represents a major target for vaccine development. This virus was first visualized and identified by LID scientists and was subsequently grown in tissue culture by workers at Merck, Sharp and Dohme. Serial cultivation in primary African green monkey kidney (AGMK) tissue culture rapidly leads to attenuation of HAV for chimpanzees as reported last year. After 10 passages in AGMK cells, HAV was observed to be highly attenuated for chimpanzees inoculated parenterally. Tenth passage HAV infected chimpanzees and induced a strong immunological response but did not cause significant disease. HAV passaged 20 times in AGMK was also attenuated for chimpanzees and retained its antigenicity. However, twentieth passage HAV remains virulent for marmosets (Saguinus mystax and Sanguinus labiatus). Infected marmosets developed a rise in serum alanine amino transferase (ALT). At this time it is not known whether the response of chimpanzees or marmosets more accurately predicts the response of susceptible individuals to tissue culture grown HAV. If the response of chimpanzees more accurately reflects that of man, currently available AGMK culture HAV should be satisfactorily attenuated for man. If the marmoset more closely resembles man in response to HAV, it will be necessary to introduce additional mutations into the virus before it can be used safely for immunization against human disease. This issue can only be resolved by cautious, stepwise trials in volunteers which are now under consideration.

HAV has been cloned biologically by three successive terminal dilutions in AGMK cells to yield a master seed that is being employed currently to prepare a twentieth tissue culture passage suspension for use in clinical trials. In addition, earlier (passage level 5) and later (passage level 26) virus stocks are being cloned by terminal dilution to yield master seed for preparation of HAV suspensions at the 10th and 30th passage level. These suspensions will be evaluated in volunteers if the 20th passage suspension proves to be over- or underattenuated. Preparation and safety-testing of HAV suspensions suitable for study in volunteers represents a collaborative effort involving RIT Laboratories, a subsidiary of Smith, Kline and French (Feinstone, Daemer, Purcell).

During the past year considerable progress was made in advancing our understanding of the molecular aspects of HAV. Previously it had been established that HAV was a picornavirus with most if not all of the characteristics of an enterovirus. The virus is a 27 nm particle with cubic symmetry that contains four polypeptides and a single-stranded linear RNA genome.

Because HAV is difficult to grow in tissue culture, a number of questions remain unanswered concerning its molecular biology. Because of the small amount of virus available, it has not been possible to define the structural features of its genome or to elucidate its genetic map. Similarly, we could not investigate genetic diversity or mode of virus replication. Recent success in cloning viral genomic sequences in E. coli using HAV RNA derived from infected marmoset liver should allow us to make significant progress in each of these areas of research. RNA was extracted from purified virions and used as a template for synthesis of double-stranded complementary DNA (cDNA) which was then inserted into a plasmid vector (pBR322) and biologically amplified in E. coli.

Poliovirus RNA was used as a model system for the evaluation of each step in the cloning of HAV RNA. Reaction conditions were identified which favored the synthesis and preservation of long cDNA copies of the scant HAV RNA available. We were most successful in producing long cDNA transcripts using HAV RNA extracted from infected marmoset livers. HAV RNA primed with oligo-(dT) served as a template for preparative cDNA synthesis with AMV reverse transcriptase. Following base hydrolysis of the RNA, a second strand of cDNA was synthesized by self-priming of the 3' hairpin loop. The cDNA was tailed with oligo (dC) and then inserted into the pBR322 vector that had been tailed with oligo (dG). E. coli HB101 was then transformed with the cDNA-pBR322 recombinants.

Characterization and identification of the recombinants included: (a) proof that cloned cDNA represented HAV sequences, (b) restriction endonuclease digestion analysis, and (c) DNA sequence analysis. To establish the identity of cloned cDNA, inserts were isolated, labelled by nick translation, and hybridized to blots of electrophoretic gels containing RNA from uninfected or HAV-infected tissue culture cells. cDNA clones hybridized to RNA from HAV-infected cells but not to RNA from uninfected cells. Genomic length RNA of approximately 7500 nucleotides was the predominant species to which cDNA hybridized. Analysis of data from restriction digests, hybridization experiments and DNA sequencing yielded a map of overlapping cDNA clones. The map spans approximately 7400 nucleotides, which represents about 99% of the HAV genome. Primer extension analysis indicated that approximately 50 bases were missing from the 5' end of the 5' clone. Heterogeneity in one restriction site was detected in a region which probably codes for a virion surface protein. A partial sequence from the 3' end of the genome contained 890 bases in an open reading frame preceding stop codons, 60 bases of a noncoding region, and a tract of poly (A). These sequences did not exhibit homology to the conserved 3' terminus of the polioviruses, indicating that these viruses have evolved separately from HAV. Sequence analysis suggests that we have cloned a heretofore uncharacterized picornaviral RNA.

It should be noted that a full cDNA copy of poliovirus is infectious when tested in tissue culture. For this reason, we will attempt to assemble cDNA clones into a full copy of the genome and test it for infectivity in tissue culture. If this effort is successful it may be possible to introduce stable deletion mutations into the cDNA form of the viral genome and evaluate their effect on virulence following conversion of HAV DNA into infectious virus. In this manner stable attenuated mutants might be produced for use in immunization. We also plan to use cloned cDNAs as reagents in other new projects, such as

detection of HAV RNA by hybridization (preliminary data from "dot blot" analysis suggests this will be a useful technique), and expression of HAV polypeptides in prokaryotic or eukaryotic cells. Finally, the nucleic acid sequence of the viral capsid proteins will allow us to deduce their amino acid sequence and this information will be used to prepare synthetic peptides that correspond to regions of potential antigenic activity. Such synthetic peptides may prove useful as immunogens, since this approach has been used successfully to immunize against foot and mouth disease virus (FMDV), another picornavirus (Ticehurst, Feinstone, Baroudy, Purcell).

Non-A, Non-B Hepatitis Viruses. In collaboration with Dr. K. Pavri of Pune, India, we are studying four outbreaks of epidemic water-borne hepatitis and two clusters of cases of endemic hepatitis in India. These occurred in Delhi in 1955, in Ahmedabad in 1975, in Pune in 1978 and in Kohlipur in 1981. None of the outbreaks could be serologically related to infection with hepatitis A virus or hepatitis B virus, although their epidemiology closely resembled that of type A hepatitis. Attempts to transmit an agent from clinical specimens from these outbreaks have been partially successful. Transient low-level liver enzyme elevations and histopathologic changes consistent with hepatitis in liver biopsies were observed in some animals, but this was not uniform, and attempts to serially transmit an agent in chimpanzees and marmosets also yielded irregular results. Characteristic histopathologic changes distinct from those seen in type A hepatitis, type B hepatitis and non-A, non-B hepatitis were reported by Dr. Hans Popper (Mt. Sinai, New York), while occasional intranuclear virus-like particles were detected by Dr. T. Kamimura (Japan), but their significance is not known (Purcell).

Other non-A, non-B hepatitis viruses play an important role in post-transfusion hepatitis and sporadic hepatitis. Although these non-A, non-B hepatitis agents cannot be detected by serologic means, they can be experimentally transmitted to chimpanzees and marmosets. These species have been useful in determining the infectivity titer of various non-A, non-B virus containing plasmas. Although most plasmas contain only 10^2 - 10^3 infectious units per ml, one plasma was found to contain over 10^6 infectious units per ml. This plasma has provided an inoculum suitable for characterization of the agent. We have recently demonstrated that at least one non-A, non-B agent contains essential lipids, a characteristic that will be important in the classification and, probably, the control of non-A, non-B hepatitis (Feinstone, Purcell).

Delta Agent. In 1977, Mario Rizzetto (a Fogarty Visiting Scientist in LID) discovered a new antigen in patients with chronic type B hepatitis. This antigen, distinct from the antigens of hepatitis B virus (HBV), was detected by immunofluorescence in hepatocyte nuclei of patients with chronic type B hepatitis. Delta was shown to be associated with a transmissible agent that required HBV synthesis for its own synthesis and that suppressed the synthesis of its HBV helper. Thus, it had the characteristics of a defective interfering agent. Delta is enclosed within a particle that is encapsidated by hepatitis B virus surface antigen (HBsAg). A 500,000 dalton segment of RNA is also enclosed, with delta, inside a coat of HBsAg. Delta was readily transmitted to chimpanzees either acutely or chronically infected with HBV but could not be transmitted to HBV-immune chimpanzees. Light and electronmicrographic changes similar to those seen during acute non-A, non-B hepatitis virus infection have

been detected in such chimpanzees, suggesting that delta may share some characteristics in common with other non-A, non-B agents.

Using a radioimmunoassay for delta and anti-delta developed by Rizzetto, it was found that delta has a worldwide distribution but is most highly endemic in Italy, especially in southern Italy, where it is associated with 50 percent of acute HBV infections. In Europe and the U.S., evidence of infection with delta is found most frequently in persons extensively exposed to blood and blood products (hemophiliacs, illicit drug users, etc.). In summary, delta appears to be a unique transmissible agent with characteristics unlike those of any previously described self-replicating agent.

Recently, in a collaborative study involving Dr. S. Hadler (CDC) and Dr. Antonio Ponzetto (Turin, Italy), serologic evidence was obtained for an etiological association between the delta agent and severe hepatitis with a high mortality in Yucpa Indians living in western Venezuela. Virtually every patient who experienced severe hepatitis had evidence of infection with the delta agent, whereas delta infection was rarely detected in HBV-positive individuals in other villages where severe disease was not observed. Death from the Venezuelan hepatitis resulted from either fulminant hepatitis or a rapidly progressive form of subacute and chronic active hepatitis.

Characterization of the agent and delta-associated hepatitis has continued using the chimpanzee model. Infectivity titrations of acute-phase chimpanzee plasma indicate an infectivity titer of delta of at least 10^{11} infectious units per ml, while the HBV titer was approximately 10^6 infectious doses per ml. Thus, we now have available an inoculum that contains delta at a titer at least 100,000-fold higher than HBV. This inoculum has permitted us to demonstrate unequivocally that delta cannot infect susceptible hosts in the absence of HBV. We are currently examining whether other viruses (e.g., HAV, non-A, non-B agents) can serve as helpers for delta replication. That viruses other than HBV can serve as a helper for delta replication was demonstrated by transmitting the delta agent from an HBV-carrier chimpanzee to a woodchuck chronically infected with the woodchuck hepatitis virus, a virus biologically similar to HBV (Purcell).

Hepatitis B virus. Hepatitis B virus (HBV) is a unique 42nm virus of complex structure that contains a double-stranded circular DNA with a single-stranded gap spanning 20-50 percent of the genome. It is the first recognized member of what is now a small group of viruses unofficially designated the "hepadna" viruses. HBV is the cause of considerable morbidity and mortality, accounting for 30-50 percent of clinical hepatitis diagnosed in the U.S. and most developed countries. However, it is in Asia and Africa that the virus has the greatest impact. Chronic infection, usually associated with hepatitis, occurs in approximately five percent of the world's population, and this may lead to death from chronic hepatitis, cirrhosis or hepatic cell carcinoma. Although relatively rare in developed countries, hepatic cell carcinoma is one of the leading causes of death from cancer in Africa and Asia, and there is considerable evidence that HBV is a causative factor in such cancer. For these reasons the control of HBV is an important public health goal.

Research in the Hepatitis Viruses Section as well as in academic and industrial laboratories elsewhere over the past decade has led to the recent

development and licensing of hepatitis B vaccines in the United States and France. These vaccines contain purified surface antigen (HBs Ag) derived from the plasma of individuals chronically infected with HBV. These vaccines have been shown to be both safe and effective in preventing Type B hepatitis, but difficulties encountered in the collection of appropriate plasma, preparation of vaccine and safety testing of the final product will limit the availability of vaccine and will make it very expensive. For these reasons, work continues on optimum formulations of vaccine and dosage regimens and on the development of "second generation" and "third generation" vaccines. These newer vaccines will probably be produced in prokaryotic or eukaryotic cells by recombinant DNA technology or they may consist of synthetic peptides.

Clinical testing and characterization of plasma-derived vaccines prepared by the NIH will soon be completed. An alum-adjuvanted preparation was found to be highly immunogenic and well tolerated in adult volunteers. Sixty-eight percent of vaccinees developed antibody within one month of vaccination and 95 percent seroconverted following completion of the six-month vaccination schedule. Several sublots have been prepared in an identical manner from the same master lot of vaccine; all have been similarly immunogenic in volunteers. The rapid antibody response to the NIH vaccine suggested that it may might be useful in preventing perinatal transmission of HBV from infected mothers to their offspring, an event that occurs frequently in Asia. In preparation for an efficacy trial of the vaccine in high-risk infants, immunogenicity tests were carried out by Dr. B. Hollinger (Baylor School of Medicine) with the NIH vaccine. The NIH vaccine was administered to 65 children 1-17 years of age. The NIH vaccine was more immunogenic in young children than in adults: 53 percent of children 11-17 years old developed antibody within two weeks of vaccination, while 78 percent of children 1-2 years of age seroconverted by two weeks. By 8 weeks, 100 percent of children 1-10 years of age had developed antibody. Reactions to the vaccine were minimal and not significantly different from those seen in recipients of a placebo preparation. Thus, the NIH vaccine was highly immunogenic and nonreactogenic for children and infants. Immunogenicity studies were carried out with the NIH vaccine in the People's Republic of China and excellent results were obtained in adults, children, infants and newborns. An efficacy trial in newborn infants whose mothers are persistently infected with HBV began in the summer of 1982. The NIH vaccine and a Chinese vaccine are being compared with a placebo in a double-blind randomized study (Purcell, Ticehurst).

In collaboration with Dr. Richard Lerner (Scripps Institute), we studied synthetic peptides that represent potential sites of HBV antigens. The amino acid sequence of these peptides was derived from knowledge of the nucleotide sequence of the HBV gene that codes for the surface antigen of the virus (HBsAg). Several peptides that represent different regions of HBsAg stimulated antibody reactive with the native surface antigen. A peptide derived from a variable region of the HBsAg gene was selected for additional studies in chimpanzees. This region was thought to define the subtype specificity of HBsAg. Three chimpanzees were vaccinated with the peptide (P-49) comprising amino acids 110-137 of the HBsAg gene product. The peptide was coupled to keyhole limpet hemocyanin (KLH) carrier and adjuvanted with alum, pertussis organisms or Freund's incomplete adjuvant. All three chimpanzees developed antibody within two weeks of vaccination, and the response appeared to be directed against the subtype specificity (γ) of the antigen. The antibody

response was relatively short-lived and attempts to "boost" the response with subsequent doses of the peptide were only partially successful. Nevertheless, the chimpanzees were challenged with live HBV to determine if the antibody that had developed was protective. Complete or partial protection of the two chimps vaccinated with alum- or pertussis-adjuvanted peptide was observed but the animal that received the Freund's adjuvant was not protected, perhaps because of the establishment of "high-dose" tolerance. These studies are being repeated with the same synthetic peptide as well as cyclic and shortened forms of this peptide (Purcell, Ticehurst).

Many tumor-bearing animals develop antibodies to unique antigens associated with the oncogenic virus causing their tumor. These nonstructural antigens, called "neoantigens," have been found in tumors caused by papovaviruses, adenoviruses, and herpes viruses. Hepatitis B virus, a hepadnavirus with suspected oncogenic potential, cannot be transmitted to non-primates but patients with HBV-associated hepatoma might be expected to have antibody to a HBV-associated neoantigen if one exists. Using a hepatoma cell line that contains integrated HBV DNA, we sought immunofluorescent antibody in sera of hepatoma patients. Approximately seven percent of sera from HBsAg-positive hepatoma patients contain an antibody that reacts with a nuclear antigen in the hepatoma cell line. This antigen was found in another hepatoma cell line that also contains integrated HBV genome but not in two other hepatoma cell lines lacking the HBV genome. The antigen is being characterized further to determine if it is the product of a transforming gene (Wen, Daemer, Purcell).

Woodchuck Hepatitis Virus (WHV). Recently, three viruses with characteristics similar to those of HBV were discovered. These are the woodchuck hepatitis virus (WHV), Beechey ground squirrel virus (BGSV), and the duck hepatitis B virus (DHB). These viruses share with HBV a similar morphology, type and organization of genome, associated DNA polymerase activity, complex of antigens (surface antigens of HBV, WHV and BGSV are antigenically related) and apparent obligate tropism for the liver. HBV and WHV are both etiologically associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts. The name "hepadnavirus" (hepatotropic DNA virus) has been suggested for this unique group of agents. None of the viruses has been isolated and propagated in tissue culture. The similarities between HBV and WHV, coupled with the tendency of each virus to cause chronic hepatitis and hepatic cell carcinoma (HCC) in their respective hosts, makes WHV and its host, the woodchuck, a particularly interesting model system. In collaboration with Dr. J. Gerin and Dr. B. Tennant, we are studying the virology and pathology of WHV-associated hepatitis and HCC in woodchucks. Sensitive radioimmunoassays for woodchuck hepatitis virus surface antigen (WHsAg) and its antibody (anti-WHs), as well as WHV core antigen (WHcAg) and its antibody (anti-WHc), were developed and these permitted us to evaluate a colony of wild-caught woodchucks for markers of infection.

To characterize the hepatitis and hepatic cell carcinoma that occur in woodchucks, a colony was established under contract with Cornell Veterinary School (Dr. B. Tennant, N.Y.S.C.V.M.). A "vaccine" containing WHsAg prepared in a manner similar to the hepatitis B vaccine, was administered to newborn animals. Other animals received a placebo. Certain of these animals were also inoculated with live WHV at birth, whereas other offspring were exposed to their WHV-positive mothers. The purpose of the study is to determine whether vaccine

can interrupt the maternal-fetal transmission of this hepadna virus in an attempt to predict the results of similar vaccine interruption trials with HBV vaccine in man. This fact is of some importance since it appears that persistence of HBV and subsequent development of HCC are favored by acquisition of infection during the neonatal period. The effect of HBV vaccine on human HCC will probably not be known for 20 to 40 years, the incubation period from infection to development of HCC. However, the incubation period to HCC in the woodchuck is approximately 2 to 5 years. Hence, important predictive information should be available from the woodchuck-WHV model system within the next few years. This information will be useful in planning for future use of hepatitis B vaccines in man.

The WHV vaccine was both safe (i.e., it did not contain infectious virus) and capable of stimulating antibody that protected against challenge with live WHV. The vaccine was also capable of preventing WHV-associated hepatitis when given to newborn woodchucks which were simultaneously administered live WHV. In contrast, placebo recipients developed hepatitis when infected with WHV at birth. Although a proportion of the vaccinated woodchucks developed serologic evidence of infection, this was inapparent and was manifest only by development of anticore antibody. Thus, experience with an inactivated WHV vaccine in newborn woodchucks predicts that vaccination of the newborn human with hepatitis B vaccine will offer significant protection against maternal-fetal transmission of HBV leading to chronic infection and HCC (Purcell).

Parvovirus-like Agents. Severe hypoplastic crisis among patients with sickle cell anemia has been associated with infection with what appears to be a noncultivable parvovirus-like agent. Immune adherence hemagglutination and radioimmunoassay techniques were adapted for the identification of this agent and its antibody. Approximately 60 percent of normal populations were found to be antibody-positive. Attempts to transmit the serum parvovirus-like agent to primates are in progress. Attempts to demonstrate replication in tissue cultures of human erythroblasts were partially successful (Mortimer, Purcell).

RESPIRATORY SYNCYTIAL (RS) VIRUS

Molecular biologic studies of RS virus. RS virus is an enveloped negative stranded virus that plays a major role in serious respiratory disease of infancy and childhood. During productive infection, the virus matures by a budding process that incorporates the cellular membrane to form its envelope. The viral envelope is studded with two different viral glycoproteins: one (G) is responsible for recognition of cellular receptors, and the other (F) is responsible for penetration and cell-to-cell spread. The latter protein, termed the fusion factor, is synthesized as a precursor that is proteolytically cleaved into two subunits (F_1 and F_2) held together by disulfide bridges. Both these proteins are immunogenic, and virus neutralization is mediated by antibodies against these antigens. One of the objectives of the RS virus research program is to overproduce these glycoproteins by recombinant DNA technology in suitable prokaryotic and eukaryotic vector systems. Glycoproteins synthesized by recombinant DNA techniques can then be evaluated to determine their efficacy as immunogens.

Recently, in order to better understand the molecular biology of RS virus, we constructed a cDNA library in *E. coli* using mRNAs from virus-infected cells. By a process of hybrid selection of viral mRNAs using recombinant cDNA plasmids and subsequent cell-free translation of the selected mRNAs, recombinants encoding the viral nucleocapsid (NC) protein, phosphoprotein (P), matrix (M) protein and a nonstructural (NS₂) protein were identified. This analysis failed to identify viral glycoprotein genes since putative precursors for these proteins were not translated regularly *in vitro* from viral mRNA(s). Occasionally, a 59 k dal protein was detected following *in vitro* translation of RS virus mRNAs. This protein was shown by other workers to be derived from a distinct viral mRNA 2240 bases in length. It has also been proposed that this protein undergoes cotranslational processing and glycosylation and it is thought to represent the precursor of the fusion factor that plays an important role in immunity.

By a process of colony hybridization, we identified several recombinant plasmids from our cDNA library that represent RS viral genes distinct from those previously identified. Successive Northern blot analysis of mRNA from infected and uninfected cells allowed us to identify two classes of recombinants, one hybridizing with a viral mRNA 2200 nucleotides in length and another with a viral mRNA 1000 nucleotides long. The larger recombinants may represent cDNA copies of mRNAs encoding the fusion factor (F). Currently we are attempting to translate viral mRNAs hybrid-selected by these recombinants. If successful, the translation products will be identified using monoclonal antibodies raised against RS viral glycoproteins (Satake, Venkatesan).

The negative-stranded, nonsegmented RNA of RS virus is approximately 5 X 10⁶ daltons. In infected cells, viral RNA always remains within a nucleocapsid structure. This property is similar to other negative-strand RNA viruses, notably the paramyxoviruses and the rhabdoviruses, of which vesicular stomatitis virus (VSV) is the prototype. The viral nucleocapsid of these viruses constitutes the basic irreducible minimum for both transcriptional and replicative functions. The transcriptionally active nucleocapsids contain the genomic RNA, a major 46 K dal nucleocapsid protein (NC), a 180 K dal polymerase (L) and an accessory protein (NS in the case of VSV and P in the case of paramyxoviruses). RS virus nucleocapsids exhibit the characteristic morphology of other negative-strand RNA viruses but they have not been shown to be transcriptionally active. Our interest in NC protein stems from a desire to understand nucleocapsid assembly during RS virus infection and as well as the various interactions between the NC protein and transcriptional enzymes. Knowledge of the primary structure of the major capsid protein should provide insight into the nature of these interactions. Toward this end, a nearly full-length recombinant cDNA representing the NC gene was sequenced. The 3' terminus of this gene was identified by the presence of 14 poly A residues at one end of the clone. The cloned DNA lacks 6 nucleotides of the 5' end of the mRNA as determined by primer extension and gene walking on viral mRNA. However, a single open reading frame of 1412 nucleotides encoding a protein of 467 amino acids is present within the cloned DNA. The protein translated from this sequence has an estimated molecular weight of 51,540 daltons and is rich in basic amino acids, relatively rich in proline, but poor in cysteine. It has no sequence homology with the capsid proteins of other negative strand RNA viruses, implying that RS virus is evolutionarily distinct. Interestingly, the sequence upstream of the poly A tail of this gene was not homologous to a similar region

in the other RS viral genes. This is unlike the situation with VSV (a rhabdovirus) and Sendai virus (a paramyxovirus), wherein a 4 nucleotide sequence upstream of poly A is conserved in all genes. It is likely that RS virus has evolved its own regulatory signals (Venkatesan, Elango).

The amino acid sequence of RS virus matrix protein was deduced from sequencing a recombinant plasmid containing a cDNA copy of this gene. The cDNA clone lacks about 10 nucleotides of the 5' end of the mRNA but the 3' end is preserved. There is one long open reading frame encoding 250 amino acids that presumably constitutes the matrix protein. There is also another open reading frame which overlaps the long open reading frame. This second open reading frame encodes a protein of 75 amino acids. Whether this second open reading frame actually produces an additional viral protein is being investigated. In this context, it should be noted that certain recombinant plasmids containing the matrix protein gene hybrid select viral mRNA(s) that yield upon translation the matrix protein and another protein that comigrates with a viral nonstructural protein (NS₂). However, it should be pointed out that these plasmids only hybridize with a viral mRNA that is 1000 bases long (Satake, Venkatesan).

Sequencing viral phosphoprotein (P) gene cDNA has just begun. Recombinant plasmids of this gene hybrid select mRNA(s) from infected cells that are translated *in vitro* to yield the phosphoprotein and a nonstructural (NS₁) protein as major products. However, by Northern blot analysis, only one mRNA about 950 bases long appears to hybridize with this recombinant plasmid. This situation differs from that of Sendai virus (a paramyxovirus), which has a nonstructural protein (C protein) that is translated from a separate mRNA encoded by sequences that overlap or are closely downstream from its P gene. Our efforts in this area will be directed toward determining the available open reading frames within the cloned P gene recombinant. We will also employ immunological methods to identify any nonstructural proteins encoded by this gene. Subsequently, biochemical analysis of infected cells may yield information regarding a possible regulatory role for the nonstructural protein (Satake, Venkatesan).

RS virus has been reported to encode 2 or 3 nonstructural proteins. At least one of these (NS₂) was shown by us to be encoded by a distinct transcript. We identified several independent cDNA clones that hybrid selected mRNA that was translated *in vitro* to yield NS₂ as its sole translation product. A cDNA insert from the largest recombinant plasmid encoding this gene was sequenced. The cDNA insert has approximately 1050 bp of RS viral sequence and is about 350 bases larger than the mRNA encoding NS₂, as determined by Northern blot analysis. Analysis of the DNA sequence revealed two different nonoverlapping open reading frames, encoding polypeptides with approximately 133 and 129 amino acids. In one reading frame, translation starts about 50 nucleotides from 5' end of the messenger strand and stops about 400 nucleotides downstream. In this reading frame there is an untranslated sequence of about 580 nucleotides between the stop codon and the poly (A) tail. In the other reading frame translation starts about 570 nucleotides from the 5' end and ends 387 nucleotides downstream, 90 nucleotides short of the poly (A) tail. Current efforts are directed at identifying which region of the cloned RS cDNA codes for the NS₂ protein and determining whether the other reading frame is translated into a viral-specific product (Elango, Venkatesan).

Biologic studies of RS virus. Previously we observed that resistance to infection of the lungs by RS virus was transferred from immune cotton rats to their non-immune parabiotic partners. Passive transfer experiments suggested that serum, rather than cells, mediated this protective effect; cotton rats pretreated with cotton rat or human serum containing RS virus antibodies were completely resistant to pulmonary infection. Duplication of this effect using purified human IgG identified serum antibody as the effector. Human antiserum (or purified IgG) administered to cotton rats after RS virus infection resulted in complete or near-complete clearance of pulmonary virus, suggesting a potential technique for specific treatment of established RS virus disease.

Potentiated disease that developed during RS virus infection of individuals previously given a formalin-killed RS virus vaccine (1966-1967) remains unexplained. Using the cotton rat, we have developed an experimental model for examining vaccine-induced potentiation. Early results suggest an Arthus-type reaction. RS virus was inoculated into a second strain of cotton rats, in which it produced overt signs of disease for the first time. Efforts are under way to establish a colony of this strain of cotton rat.

Earlier efforts to develop a vaccine for RS virus were directed primarily at genetic alteration of wild-type human RS virus. To date none of these efforts has proven successful. Recently an avian tissue culture-adapted strain of human RS virus (HRSV), thought to be attenuated, was tested in chimpanzees; unfortunately, it was observed to be fully virulent. Previous investigations of bovine RS virus (BRSV) were extended; and six strains have now been evaluated in cotton rats. Several show promise in protecting animals from HRSV infection. Further evaluation in primates is contingent upon development of a technique for sampling the lower respiratory tract for RS virus by pulmonary lavage (Prince).

INFLUENZA VIRUSES

Genetics of attenuation. We had previously characterized ten avian influenza A viruses for growth in squirrel monkeys in an effort to identify a virus that was attenuated for primates and that could be used as a donor of genes that would attenuate virulent human influenza A viruses by genetic reassortment. Several such attenuated avian viruses were identified. Subsequently one such virus, the avian A/Mallard/6570/78 (H2N2) influenza virus, that was 1000 times restricted in replication in the upper and lower respiratory tract of squirrel monkeys, was mated with a virulent human influenza A/Udorn/72 (H3N2) virus. From this coinfection, a reassortant virus that contained the hemagglutinin and neuraminidase genes of the virulent human virus and the six "internal" genes of the attenuated avian virus was isolated. This reassortant was attenuated and immunogenic in squirrel monkeys. Furthermore, the reassortant virus did not produce a systemic infection; it remained localized to the respiratory tract. It was also stable with regard to phenotype (i.e., it was restricted in replication in the respiratory tract) during 5 successive passages in monkeys.

Our experience with the avian (A/Mallard/78)-human influenza reassortant indicated that the genetic determinants of attenuation of this avian influenza A virus reside on one or more of its "internal" genes (i.e., genes that code for nonsurface viral proteins). In the past year we sought to determine if the attenuated A/Mallard/78 (H2N2) virus would regularly render current epidemic

H1N1 and H3N2 human influenza A viruses attenuated for primates by transfer of its six "internal" avian influenza genes. Two avian-human influenza reassortant viruses possessing the A/Washington/80 (H3N2) or A/California/10/78 (H1N1) hemagglutinin and neuraminidase genes plus the six "internal" avian influenza genes were produced by coinfection at 42°C, a temperature restrictive for human influenza "internal" genes but permissive for avian influenza "internal" genes. Each reassortant exhibited the same shutoff temperature for plaque formation (42°C) as the parental avian influenza virus. In addition, each reassortant was as attenuated as its avian influenza parent in squirrel monkeys and hamsters. Preliminary evaluation in volunteers revealed both viruses to be attenuated and immunogenic. These observations indicate that influenza A reassortant viruses containing the six "internal" avian influenza genes plus human influenza HA and NA genes can be generated readily *in vitro*. Furthermore, the transfer of these avian influenza genes reproducibly confers attenuation on the resulting avian-human influenza virus reassortant (Murphy, Chanock).

To identify the genetic determinants of attenuation of the avian A/Mallard/78 virus, reassortant viruses were produced that contained the HA and NA genes of the human A/Udorn/72 virus and one or more of the internal genes of the A/Mallard/78 (H2N2) parent. These reassortants were evaluated for their efficiency of plaque formation (eop) at different temperatures and their level of attenuation for monkeys. Reassortant viruses bearing only the RNA 1, RNA 3 or NS RNA of the avian influenza virus parent did not exhibit restriction of growth in monkeys. However, two reassortants containing all human influenza virus genes except an avian influenza virus M or NP gene were as attenuated in monkeys as their avian influenza virus parent. These observations suggest that the avian influenza NP and M genes play a major role in restriction of viral replication in primates.

The "internal" genes of two additional avian influenza viruses were shown to determine restriction of viral replication in primates. Avian-human reassortant viruses were produced by mating the avian influenza A/Mallard/Alberta/503/78 (H1N1) or A/Pintail/Alberta/121/79 (H7N8) virus with the human influenza A/Udorn/72 (H3N2) virus. Reassortant viruses containing the hemagglutinin (HA) and neuraminidase (NA) genes from the human influenza virus parent and the other six RNA segments from the avian influenza parent were then evaluated for their level of replication in the upper and lower respiratory tract of squirrel monkeys. The reassortants exhibited the same degree of restriction of growth in the respiratory tract as their avian influenza parent. These findings have implications for the use of avian influenza A viruses as donors of "internal" genes for attenuation of new epidemic or pandemic human influenza A viruses. It appears that attenuation of human influenza A viruses by transfer of "internal" genes from a restricted avian influenza A virus may be a general phenomenon. By evaluating a variety of avian influenza viruses that differ in their level of replication in primates, it should be possible to identify one or more viruses whose "internal" genes regularly confer the most satisfactory balance of attenuation and immunogenicity upon avian-human influenza virus reassortants (Murphy, Tian, Buckler-White, Chanock).

Evaluation of avian-human influenza virus reassortants in volunteers. The human A/Washington/80-avian A/Mallard/78 reassortant virus containing the HA and NA genes of the virulent human influenza virus and the six "internal" genes of the avian influenza virus was administered at a dose of $10^{8.0}$, $10^{7.5}$, $10^{7.0}$, $10^{6.6}$,

or $10^{5.0}$ TCID₅₀ to 86 seronegative adult volunteers. Febrile illness was not observed at any dose and only two systemic reactions were seen, both at a dose of $10^{7.0}$ TCID₅₀. The infectivity data is preliminary, but at $10^{7.0}$ TCID₅₀, 16 of 19 volunteers were infected, and at $10^{8.0}$ each of 10 volunteers volunteers was infected. Virus was shed for a short duration (mean of less than a day) and in low quantity (mean titer of less than $10^{1.0}$ TCID₅₀). The serum and nasal wash immune responses were equivalent to those seen with ca reassortant viruses. These observations indicated that the avian-human influenza reassortant virus was satisfactorily attenuated and antigenic in susceptible adult volunteers (Murphy).

Evaluation of cold-adapted (ca) influenza virus reassortants

in volunteers. One hundred and thirty-one susceptible adult volunteers were given an A/Wash/80 ca reassortant virus possessing the 6 "internal" ca genes of the A/Ann Arbor/6/60 donor and the hemagglutinin and neuraminidase genes of the virulent A/Wash/80 virus. Virus was administered intranasally at a dose of $10^{5.5}$, $10^{6.0}$, $10^{6.5}$, $10^{7.0}$ or $10^{7.5}$ TCID₅₀. The human infectious dose₅₀ (HID₅₀) was between $10^{5.0}$ to $10^{6.0}$. The reassortant was satisfactorily attenuated; only 3% of volunteers developed an afebrile systemic reaction. In contrast, the virulent influenza A/Wash/80 parent induced a febrile and/or systemic response in 38% of volunteers. The ca reassortant was nontransmissible and stable as regards the ts and ca phenotypes. Similar observations were made with an influenza A/Calif/10/78 (H1N1) ca reassortant. Our experience indicates that transfer of the six internal genes of the A/AA/6/60 (H2N2) cold-adapted parent virus reproducibly confers a desirable set of properties on human influenza A H1N1 or H3N2 reassortant viruses: (1) satisfactory level of attenuation; (2) immunogenicity; (3) lack of transmissibility; and (4) stability of ca and ts phenotypes (Murphy).

Comparison of the influenza A/Wash/80/ ca reassortant given intranasally with inactivated influenza vaccine given intramuscularly indicated that the former induced greater resistance to challenge with virulent influenza A/Wash/80 virus. Of interest, the inactivated vaccine stimulated a greater serum antibody response, while infection with the ca reassortant induced a greater local antibody response. Clearly, immunity induced by live attenuated virus cannot be predicted by immunologic criteria used to assess parenteral vaccination with inactivated antigen (Murphy).

Immunogenicity of synthetic peptides. Preliminary experiments were performed in hamsters to evaluate the immunogenicity of synthetic peptides corresponding to major portions of the influenza A/Victoria/3/75 hemagglutinin (H3), including the known antigenic sites as well as the N' terminus of the HA-2 subunit. The synthetic peptides were conjugated to KLH and administered in Freund's complete adjuvant. These studies were initiated in collaboration with Dr. Richard Lerner and Dr. N. Green of Scripps Institute, La Jolla, California. The hamsters developed antibodies that bound to purified hemagglutinin or whole virus, but these antibodies lacked hemagglutination-inhibiting and neutralizing activities. Hamsters immunized with synthetic peptides exhibited little or no resistance to challenge with wild type virus, whereas hamsters infected previously with influenza A/Victoria/3/75 virus or inoculated previously with inactivated whole virus vaccine resisted challenge (Murphy, van Wyke, Chanock).

Immunologic characterization of influenza virus proteins using monoclonal antibodies. In contrast to the extremely variable surface proteins of influenza viruses, the internally located matrix protein has been characterized as being antigenically invariant. Since monoclonal antibodies produced to the influenza nucleoprotein have enabled us to study in detail antigenic variation of this protein in influenza viruses and to define topographical domains of the nucleoprotein involved in RNA transcription, we chose this approach to also study the matrix protein. These studies were done in collaboration with Dr. Jonathan Yewdell at the Wistar Institute. Five monoclonal antibodies were produced to the WSN/33 (H1N1) strain of influenza A virus, and their specificities for the matrix protein were demonstrated by radioimmunoprecipitation. When tested in enzyme-linked immunosorbent assays (ELISA) against a panel of influenza A viruses representing the major surface antigen subtypes, three unique reactivity patterns were obtained. This indicated that (1) antigenic variation occurs in the matrix protein of influenza viruses, and (2) this variation involves a minimum of three antigenic sites. The antigenic variation observed is characteristic of the phenomenon of genetic polymorphism. Competitive-binding ELISA of these three antibodies indicated that they defined at least two non-overlapping antigenic domains. Since antibodies to the third antigenic site exhibited non-reciprocal competitive binding in ELISA, it was possible that this antigenic site partially overlapped one of the other antigenic sites under study. To resolve this question, purified M protein was subjected to limited proteolysis, and the fragments were probed by the 3 monoclonal antibodies in Western blot analysis. The results of this assay demonstrated that the antibody in question indeed recognized a third non-overlapping antigenic region of the matrix protein. In contrast to monoclonal antibodies to the nucleoprotein, none of the matrix antibodies inhibited in vitro RNA transcription (van Wyke).

Monoclonal antibodies to four distinct antigenic sites on the hemagglutinin of the A/Udorn/307/72 (H3N2) strain were produced for the purpose of antigenic characterization of the hemagglutinin. The gene coding for this protein had been cloned and expressed in a SV40-HA recombinant in African green monkey cells by Dr. C.J. Lai. Two forms of the protein were compared for reactivity with the monoclonal antibodies in fluorescent antibody binding assays: the first was the complete protein in its glycosylated form and the second was a complete nonglycosylated protein produced by a mutant gene with a deletion in its leader sequence. Both glycosylated and nonglycosylated forms of the genetically engineered hemagglutinin retained all four antigenic sites which are found on the virion hemagglutinin (van Wyke).

MOLECULAR BIOLOGIC ANALYSIS OF INFLUENZA VIRUS

Transcriptional control. Following infection of a permissive cell, influenza viral cytoplasmic mRNA's are transcribed from the virion RNA segments. In collaboration with Dr. Robert Lamb, it was shown previously that both uninterrupted (colinear) and interrupted mRNA species are produced from viral RNA segments coding for the matrix protein (M) and non-structural proteins (NS). To demonstrate that interrupted mRNA's are generated by splicing, cloned influenza NS cDNA was inserted into an SV40 vector so that the late SV40 transcription signals directed the synthesis of (+) strand NS RNA sequences. Poly A-containing RNA from African green monkey kidney (AGMK) cells infected with the SV40-NS recombinant was analyzed for the presence of NS sequences. A

colinear and an interrupted NS RNA species were detected. The interrupted NS RNA contained a splice sequence at the juncture similar to that of NS₂ mRNA produced during influenza virus infection. The NS polypeptides produced by the SV40-NS recombinant were then analyzed using immunoprecipitation and gel electrophoresis. As predicted, the the NS₁ and NS₂ proteins of influenza virus were detected. Specific splicing from the transcript of SV40-NS DNA ruled out the possibility that influenza mRNA's are formed by "jumping" of the influenza virus polymerase at the consensus sequences during replication. The presence of colinear as well as spliced NS mRNA's establishes that processing occurs during influenza virus transcription in vivo (Lai).

Deletional analysis of the influenza virus neuraminidase (NA). A full-length ds DNA copy of an influenza A N2 neuraminidase (NA) gene was cloned into the late region of pSV2330, a hybrid expression vector that includes pBR322 plasmid DNA sequences, the SV40 early region and the SV40 late region mRNA intervening sequences thought to stabilize late mRNA transcripts. The product of the cloned wild-type NA gene was shown to be present in the cytoplasm of fixed cells and at the surface of "live" (unfixed) cells by indirect immunofluorescence using N2 monoclonal antibodies. Immunoprecipitation of ³⁵S-methionine labeled proteins from wild-type vector-infected cells using heterogeneous N2 antibody showed that the product of the cloned NA DNA comigrated with glycosylated NA from influenza virus infected cells, remained associated with the membrane fraction, and formed an immunoprecipitable dimer. Using a low molecular weight substrate for the NA that releases a fluorescing moiety upon hydrolysis, NA enzymatic activity was detectable after SV40 lysis of vector-infected cells. These properties of the product of the cloned wild-type gene were compared to those of the polypeptides produced by three deletion mutant NA DNAs that we constructed and then cloned into the late region of the pSV2330 vector. These mutants lacked 7 (d1 k), 21 (d1 I) or all 23 amino acids (d1 Z) of the amino-(N-) terminal variable hydrophobic region thought to anchor the mature wild-type NA tetrameric structure in the outer membrane of the infected cell as well as in the influenza viral envelope. Comparison of the phenotypes of these mutants showed that this region in the NA molecule functions not only in membrane anchorage but also as a signal sequence, permitting entry of the nascent NA polypeptide into membrane organelles for glycosylation (Markoff, Lai)

Deletional analysis of the influenza virus hemagglutinin (HA). Requirements for cell surface expression of the influenza hemagglutinin (HA) were studied using a recombinant of SV40 which had full-length DNA sequences coding for the influenza virion HA inserted into the late region of SV40. The recombinant was propagated in the presence of an early ts A mutant of SV40 that served as helper. Infection of primate cells with the SV40-HA recombinant produced a functional glycosylated HA polypeptide that accumulated on the cell surface. To delineate the protein domains necessary for surface expression of the HA polypeptide, mutations were engineered in the recombinant SV40-HA DNA. One mutant of interest sustained a deletion of 5 base pairs at the Bam HI site generating a shift in reading frame for the carboxy-terminal 37 amino acids. This region includes a hydrophobic sequence of 24 amino acids that is followed by the 13 carboxy-terminal amino acids. The shift in reading frame yielded a terminal sequence in which 21 of the first 24 amino acids remained hydrophobic or neutral, while the remainder of the carboxy-terminus contained 4 charged amino acids. Although its carboxy-terminus resembled wild-type topologically, the mutant HA was glycosylated but was not secreted or expressed on the cell surface, but instead

remained intracellular. These findings suggest a role for the hydrophobic carboxy-terminus in cell surface expression (Sveda, Lai).

Wild-type SV40-HA recombinant cloned at the unique Bam H1 site of pBR322 was used for the derivation of other HA deletion mutants with alteration of signal peptide sequences at the amino-terminus. Deletion mutations were introduced at a specific MboII site located 12 base pairs downstream of the initiation codon of HA. One mutant was of particular interest because it sustained an in-phase deletion. This in-phase mutant HA sustained a deletion of 33 bp or 11 amino acids, all within the signal peptide. Sequence analysis predicted that 5 amino acids of the 16 amino-acid signal sequence remained as a result of this deletion. The signal peptide cleavage site of Gly-Gln and the subsequent downstream sequences were not affected. Mutant HA lacking 11 amino acids of signal sequence accumulated in the cytoplasm but was not inserted into the outer membrane. The mutant polypeptide synthesized during infection was not modified by glycosylation. Its failure to agglutinate red blood cells suggests that the mutant HA remained monomeric since the trimeric structure is required for functional activity. This functional defect of mutant HA may be due to the lack of carbohydrate components which are required for proper assembly (Sekikawa, Lai).

Attempts to rescue cloned influenza virus DNA by allele replacement. Recently, cloned, full-length DNA derived from the single stranded RNA genome of poliovirus was shown to be infectious by Racaniello and Baltimore. This means that mutations introduced at strategic locations in full-length poliovirus cDNA can now be studied for their effect on viral structure, function and virulence. Similarly, our goal with influenza virus is to devise procedures that would permit conversion of influenza DNA to influenza virus genomic (negative strand) RNA and then transfer such RNA into an influenza virus. In this manner, stable site-specific mutations, such as deletions, induced in the cloned DNA, might be transferred back to the influenza virus. It might then be possible to develop stable mutants for experimental study and for use in immunoprophylaxis.

To determine whether cloned influenza DNA could be converted back to viral RNA (vRNA) and packaged in the virion, influenza gene rescue experiments (so-called allele replacement) were attempted employing the following protocol. Recombinant SV40-HA and SV40-NA DNA were used because of the ease of identification of the two surface antigens. Also specific antiserum which effectively neutralized virus bearing HA or NA of the coinfecting virus could be used to facilitate detection of reassortant viruses that had undergone allele replacement. Permissive African green monkey kidney cells were infected sequentially with a SV40-HA (H3) or SV40-NA (N2) recombinant followed by influenza A/WSN/1933 (H1N1) which bore surface antigens of another subtype. Infected cell lysates were passaged once and incubated with WSN antiserum to neutralize progeny virus bearing H1 or N1 and thus favor the detection of reassortant virus that had undergone allele replacement. Heterogeneous WSN antiserum (anti-H1N1) was used in the attempt at allele replacement of the H1 gene, while monoclonal antibody capable of neutralizing virus bearing N1 was used in the attempt at replacement of the N1 gene. This protocol should allow detection of reassortant virus that had acquired an RNA segment derived from a HA or NA DNA insert resulting in replacement of the corresponding WSN gene. When SV40-HA or SV40-NA was tested in this manner, rescued virus could not be identified. Using SV40-NA recombinants which produced either a positive strand

or a negative strand RNA transcript, we were unsuccessful in detecting rescued virus. Positive full-length influenza RNA sequences were produced from the former orientation, but they were flanked at both ends by SV40 sequences (5' late SV40 transcription initiation sequences and 3' polyadenylation signals). It is possible that the viral replicase provided by the coinfecting influenza virus did not recognize the terminal influenza sequences because of these flanking SV40 sequences and was thus unable to start influenza vRNA synthesis. Similarly, failure to rescue negative RNA transcripts produced during infection by SV40-HA or SV40-NA with an influenza insert in the opposite orientation, suggests that influenza transcripts presumably containing flanking SV40 sequences were not encapsidated, replicated and packaged in virions. Precise terminal sequences may be a prerequisite for transcription, replication, and encapsidation of full-length influenza RNA. If this is the case, the 5' and 3' flanking SV40 sequences contained in RNA transcripts from the recombinant influenza DNA may have prevented rescue. In order to provide specific terminal sequences in the RNA transcripts that are recognized by the influenza replicase complex, it may be necessary to remove the flanking SV40 sequences (Lai, Markoff, Sveda, Chanock).

Persistent expression of influenza virus nucleoprotein (NP) in eukaryotic cells. Selective complementation of defective influenza A virus mutants and ultimately, "rescue" of cloned mutant influenza DNA into influenza virions may require the expression of cloned genes in persistently infected, stably transformed cells. For example, one obstacle to achieving complementation and rescue during lytic infection by our established SV40 vectors is interference of co-infecting influenza virus by replicating SV40. Bovine papilloma virus (BPV) is a large DNA virus that replicates autonomously and extrachromosomally during persistent infection of animal cells. Recently a collaborative effort was initiated with Drs. Peter Howley and Ming-fan Law (NCI) in an effort to exploit their BPV vector for the expression of cloned influenza viral genes. A BPV recombinant that incorporated the influenza nucleoprotein (NP) gene was successfully constructed.

The BPV-NP recombinant DNA was used to transform mouse C127 cells. Infected cells were analyzed for the production of the NP protein by (1) polyacrylamide gel electrophoresis in order to estimate molecular size, (2) indirect immunofluorescence in order to establish the location of NP protein in infected cells and (3) labelling with ³²P orthophosphate in order to detect post-translational modification of NP protein. It was shown that (1) protein from BPV-NP transformed cells was immunoprecipitated specifically by NP monoclonal antibodies; (2) a labelled protein of 56K daltons equivalent in size to the NP produced during influenza virus infection was produced in BPV-NP infected cells, while such a protein was not formed in BPV transformed cells; (3) the NP protein localized in the nucleus and cytoplasm of BPV-NP transformed cell; (4) the NP protein was also phosphorylated in the BPV-NP transformed cell.

The mouse cell line (C127) used in the initial studies is not permissive for influenza virus replication and hence can not be employed for gene rescue ("allele replacement"). For this reason, other host cell systems were examined to determine if they could support both influenza virus replication and transfection by BPV. Simian CV-1 cells show some promise in this regard. CV-1 cells were co-transfected with the BPV-NP recombinant and a neomycin resistance gene cloned in the pSV-2 vector. Neomycin selection was then used to facilitate

detection of NP protein expressed in cotransformed neomycin resistant CV-1 cells. Using indirect immunofluorescence for detection of antigen it was observed that the NP protein was synthesized in a small percentage (approximately 1%) of neomycin resistant cells. Currently single cells are being cloned in order to obtain a BPV-NP transformed cell line that will persistently express NP protein. It should be noted that neomycin-resistant CV-1 cells were found to be permissive for influenza virus infection.

Cell lines persistently expressing a cloned influenza gene should be suitable for the selection of viral mutants with mutations on the gene corresponding to the cloned influenza gene that is persistently expressed. In this manner it may be possible to select stable mutant genes that can be used to confer attenuation on epidemic or pandemic strains of influenza virus by gene reassortment (Lin, Lai).

VIRAL GASTROENTERITIS

Small 27nm viruses that cause acute, epidemic, nonbacterial gastroenteritis. A variety of 27 to 32 nm viruses have been shown to be etiologically associated with epidemic nonbacterial gastroenteritis. The Norwalk virus is the most thoroughly studied member of this group. Seropidemiologic studies using a sensitive solid phase radioimmunoassay (RIA) indicate: (1) Norwalk virus has a world wide distribution, (2) Norwalk infection is responsible for at least one-third of outbreaks of nonbacterial gastroenteritis in the U.S., and (3) the majority of Norwalk outbreaks are waterborne but some are associated with contaminated food or shellfish. Biophysical characterization of Norwalk virus indicates that its proteins resemble those of caliciviruses.

Recently a serologically distinct 27 nm gastroenteritis virus, the Marin County virus, was successfully transmitted to adult volunteers. One volunteer shed large amounts of virus which was purified by ultracentrifugation techniques and used to make hyperimmune antiserum. This antiserum was then used to develop a sensitive and specific RIA. Studies to date indicate that the Marin County virus is not a frequent cause of epidemic nonbacterial gastroenteritis. Purified virus was characterized and shown to have 1 or 2 structural proteins (30 Kd); thus, Marin County virus does not resemble a picornavirus or a calicivirus. Monoclonal antibodies to purified Marin County virus are currently being isolated (Greenberg, Midtun).

Cultivation and antigenic characterization of human and animal rotaviruses. Rotaviruses are a major cause of serious diarrheal disease of infancy and early childhood. Understanding these viruses and the extent of their antigenic diversity has been a major goal of our enteric virus disease program. Towards this end, over 80 strains of human rotavirus were cultivated in heteroploid MA104 or primary AGMK cells. Four distinct serotypes of human rotavirus were identified. These serotypes were then compared with each other and with 12 animal rotavirus strains. Three additional distinct serotypes were defined and these were found only among the animal rotavirus strains tested; however, 6 animal rotaviruses were found to be antigenically similar if not identical to human rotaviruses belonging to serotype 3 or 4. Animal rotaviruses related to human serotypes 1 and 2 were not identified. Potential candidate

vaccine strains of different serotypes have been grown either in primary or secondary African green monkey kidney or in diploid cells which are suitable for vaccine production. These virus preparations will be evaluated further for safety and efficacy (Wyatt, Hoshino).

Experimental rotavirus studies in animals. Gnotobiotic piglets and newborn rhesus monkeys were studied to evaluate virulence of selected human and animal rotavirus strains as well as to study homologous and heterologous immunity. Three potential vaccines or vaccine precursors [human rotavirus strain D (serotype 1), bovine rotavirus UK and rhesus rotavirus (serotype 3)] were evaluated in gnotobiotic piglets. Both the human D strain rotavirus and the rhesus rotavirus infected piglets but neither caused dehydrating diarrhea. The UK bovine rotavirus did not cause disease, which was not surprising, since it produced little, if any, infection. A human rotavirus belonging to the 4th serotype infected gnotobiotic piglets without causing dehydrating diarrheal illness. Prior infection of piglets with rhesus rotavirus (serotype 3) induced demonstrable resistance to subsequent challenge with a human rotavirus belonging to the same serotype. This effect was manifest by decreased shedding of the human rotavirus following challenge. Rhesus rotavirus infected juvenile rhesus monkeys but did not produce disease. Additional safety testing of this strain to rule out the presence of an agent which produces acquired immune deficiency in rhesus monkeys must be successfully completed before this promising vaccine candidate strain can be evaluated in volunteers (Wyatt, Hoshino).

In a previous study of heterotypic immunity, we demonstrated that in utero inoculation with bovine rotavirus protected calves against challenge with a human rotavirus belonging to a different serotype. Additional evaluation of the calf sera and fecal samples from this study indicated that a single in utero exposure to bovine rotavirus induced broadly reactive neutralizing antibody and significantly decreased viral shedding following subsequent challenge with human rotavirus (strain D). Heterotypic antibody may explain the observed heterotypic immunity (Wyatt, Hoshino).

Studies of rotaviruses in volunteers. Rotaviruses are a major cause of diarrhea in infants and young children. It appears that an effective rotavirus vaccine would not only reduce morbidity from diarrheal diseases in the developed countries but would also reduce mortality from these diseases in the developing countries. With the successful adaptation of human rotaviruses to cell cultures a major obstacle to the development of a vaccine has been overcome. It remains, however, to identify or develop one or more rotaviruses that induce resistance without causing unacceptable clinical signs or symptoms of illness. Studies leading to the identification of such potential vaccine candidates are carried out initially in animals in which safety, virulence, and immunogenicity are evaluated. The first test of attenuation and immunogenicity in man is then performed in adult volunteers. Afterward, efficacy studies are performed during which immunized volunteers are challenged with virulent rotavirus. Before embarking on studies in volunteers it was necessary to gain an understanding of the correlates of resistance to rotavirus disease. This knowledge was achieved in part and this enabled us to select susceptible volunteers for studies with candidate vaccine strains.

During a previous study, we observed that wild-type human rotavirus, strain D (serotype 1), induced diarrheal illness in adult volunteers with low levels of

serum antibodies. During the course of this study we identified serologic correlates of resistance. In a subsequent study, human rotavirus, Wa strain (serotype 1), adapted to growth in African green monkey kidney cell cultures was shown to infect susceptible volunteers. The failure of adult volunteers with a low level of pre-challenge serum antibody to develop diarrheal disease suggested that the Wa tissue culture-adapted rotavirus mutant was attenuated compared to the wild-type D strain rotavirus. The suspension of Wa strain employed in these studies cannot be used in future studies because a simian foamyvirus was recently identified in the seed virus used to prepare it. Also 3 volunteers developed a low-level rise in serum transaminase 10 days after administration of virus; the etiology of these rises has not been explained as yet. Because of these problems, an additional suspension of the Wa human rotavirus is being prepared in pre-tested, adventitious virus-free African green monkey kidney cell cultures. It is planned that this virus suspension will be evaluated in susceptible adult volunteers. Efforts are also under way to adapt human rotaviruses to growth in human or rhesus monkey diploid cells; if successful, vaccines could be prepared in these virus-free cells (Wyatt, Kapikian, Chanock).

Rotavirus genetics--use of gene reassortment to establish gene coding assignments and preparation vaccine candidate strains. Rotaviruses, as members of the reoviridae have a segmented double-stranded RNA genome. Like reoviruses and myxoviruses, they undergo gene reassortment at high frequency during mixed infection. Advantage was taken of this property to produce and isolate rotavirus reassortants to study basic properties of the rotaviruses, i.e., the phenotype-genotype relationship of several important viral functions. In addition, reassortants were isolated from primary African green monkey kidney (AGMK) cells coinfecting with a wild type animal rotavirus (bovine or rhesus) and a human strain belonging to each of the 4 distinct serotypes. These reassortants were serotypically human but contained mostly animal rotavirus genes (10 in several instances). Because of the narrow and highly restricted host range observed for rotaviruses recovered from a wide variety of species it is likely that substitution of a large number of animal rotavirus genes for the corresponding genes of a human rotavirus will lead to attenuation for man. These reassortants are currently being prepared for study in experimental animals and ultimately in man.

Bovine x Wa (human serotype 1), bovine x DS-1 (human serotype 2), and bovine x W (human serotype 1) reassortants were analyzed in order to establish gene coding assignments for subgroup antigen specificity, neutralization antigen specificity, and growth restriction of human rotaviruses in tissue culture. During these studies it was observed that the neutralization antigen and subgroup antigen specificities reassorted independently. Gene 4 is associated with restriction of growth of human virus in vitro, while gene 6 codes for the protein that carries subgroup antigen specificity. Depending on the virus, either gene 9 (Wa or W) or gene 8 (DS-1) codes for neutralization antigen specificity. A series of reassortants derived from the growth yield of mixed infection with ts bovine rotavirus x ts rhesus rotavirus (RRV) were also studied to increase our understanding of the organization of the rotavirus genome. The genes coding for the primary neutralization antigen (serotype) and the viral hemagglutinin reassorted independently. In addition, we found that protease enhanced plaque formation in tissue culture co-segregated with the viral hemagglutinin. Analysis of the genotypes of the bovine (UK) x RRV reassortants

demonstrated that the viral hemagglutinin and protease enhanced plaque formation were coded for by gene 4, while the serotype protein was coded for by either gene 8 or 9. In addition, analysis of phenotypes of the UK x RRV reassortants demonstrated that virus neutralization was also mediated at a lower level by antibody to the gene 4 product. The demonstration that the viral hemagglutinin and neutralization proteins were distinct was a surprise, since this is not the case with the reoviruses which resemble the rotaviruses in most basic properties.

In the last year we succeeded in isolating a large number of reassortant viruses derived from coinfection of noncultivable human rotavirus (serotype 1, 2 or 3) and wild type bovine or rhesus rotavirus. These reassortants were specifically isolated as vaccine candidate strains. Wild type animal rotaviruses were used since these viruses are less likely to have silent point mutations than mutagenized ts virus. Silent single point mutations are generally unstable and tend to confound genetic analysis of attenuation. In order to isolate reassortants, two selection processes were used. First, noncultivable human strains were employed. Second, antiserum specific for the animal rotavirus 38Kd glycoprotein ("neutralization antigen") was used for selection of reassortants with the desired human rotavirus antigenic specificity. Using this strategy, reassortants of 3 of the 4 human serotypes were isolated employing either RRV or bovine rotavirus as the animal rotavirus parent. Preliminary analysis of the genotype of these reassortants indicates that many reassortants possess 10 genes of animal rotavirus origin and only a single gene of human rotavirus origin, the human rotavirus gene that codes for the neutralization (i.e., serotype specific) protein. Such reassortants with a single human rotavirus gene substitution represent promising candidate live vaccine strains. Single gene substitution reassortants of this type should retain the growth restriction of the animal rotavirus parent in human tissues and hence be attenuated for man. On the other hand the major protective antigen would be derived from the human rotavirus parent (Greenberg, Midtun, Kapikian, Chanock).

Rotavirus proteins. In an attempt to clarify the nature of various rotavirus antigens and as a means of investigating rotavirus protein structure and function, a series of monoclonal antibodies directed at several rotavirus proteins were isolated. Monoclonal antibodies were derived from mice immunized with the Wa strain of human rotavirus (serotype 1), the rhesus strain of simian rotavirus (RRV) or a human rotavirus (DS-1, serotype 2) reassortant (40-2). A total of 70 monoclonal antibodies directed at 6 viral proteins were identified. Monoclonal production utilized standard techniques and assay systems including RIA, neutralization, immunoprecipitation and hemagglutination-inhibition (HI). The monoclonals were identified by the proteins with which they reacted. These included: (1) monoclonals to the 42,000 dalton inner protein, the 6th gene product; two of these exhibited subgroup antigen specificity. (2) monoclonals to the 34,000 dalton surface glycoprotein, the 8th or 9th gene product; some of these neutralize virus to high titer and some exhibit HI activity. (3) monoclonals to the 82,000 dalton surface protein, the 4th gene product (i.e., the viral hemmagglutinin); these monoclonals inhibit hemagglutination and neutralize virus. (4) monoclonals to the 28,000 dalton 10th gene product, the nonstructural glycoprotein; (5) one monoclonal that reacts with the 35,000 dalton NS glycoprotein and finally (6) several monoclonals that cannot be classified.

These monoclones have proved to be helpful in establishing or confirming gene-product relationships (Greenberg, Midtun, Walsh).

Genetic diversity of rotaviruses studied by nucleic acid hybridization. The ability of rotaviruses to transcribe their genomic RNAs "in vitro" into single stranded (ss) RNAs was used to prepare radioactive probes. These probes were then employed in hybridization assays for detection of rotaviruses and for the analysis of the genetic relatedness among isolates of human and animal origin.

A dot hybridization assay using (ss) RNA probes permitted unequivocal recognition of rotavirus in stool and in tissue culture material. The method is highly specific and allows the detection of as little as 8 pg of viral RNA. A high degree of concordance was found between the dot hybridization assay and other methods used for detection of rotaviruses such as ELISA, electron microscopy or RNA analysis. Its sensitivity is great enough to allow the detection of rotavirus in rectal swabs obtained from children with diarrhea (Flores).

Radioactively labeled RNA probes have also been used in the analysis of genetic relatedness among human and animal rotavirus strains. For these assays, genomic RNA from various viruses was heat-denatured and hybridized to (ss) RNA probes prepared from other rotavirus strains. The resulting hybrids were analyzed by electrophoresis and autoradiography. In an initial study, it was found that although a high degree of heterogeneity existed in the RNA electrophoretic patterns of rotaviruses isolated from children with diarrhea in the same hospital on the same day, each of the specimens hybridized significantly to a probe prepared from the prototype human strain Wa (type 1) or human strain DS-1 (type 2) but never to both probes. Based on these observations, it was suggested that there were at least two genetic "families" of human rotavirus; members of the Wa family seem to share a high degree of homology among themselves but not with the DS-1 family and vice versa.

During the past year more than 80 additional rotavirus specimens were obtained from Venezuela through the cooperation of Dr. I. Perez. Hybridization analysis of these virus strains add support to the concept that two major families of rotaviruses exist. However, several exceptions were particularly instructive: (a) RNA from two specimens failed to hybridize to either probe (Wa and DS-1); (b) two other viruses had RNAs that hybridized mainly to the Wa probe but they also formed a hybrid segment with the DS-1 probe which had a mobility similar to that of the gene triplet 7-8-9. Such exceptions suggest that there may be more than two rotavirus "families" and that gene reassortment may occur in nature between members of the Wa and DS-1 families (Flores).

Molecular cloning of rotavirus genes. Previous work from our laboratory has established that the eighth or ninth gene (in order of electrophoretic migration) of the human rotavirus strains DS-1 and Wa (respectively) codes for the main virus neutralization antigen. Cloning of DNA copies of those genes in bacteria would represent the first step towards the achievement of antigen expression in vitro. We have recently succeeded in cloning such genes from the human rotavirus strain Wa (serotype 1), the bovine Nebraska calf diarrhea virus (NCDV), and rhesus rotavirus (related to human serotype 3).

Cloning of rotavirus genes was achieved through reverse transcription of rotavirus RNAs obtained either from rotavirus particles [genomic, (ds) RNAs] or from an "*in vitro*" transcription reaction [(ss) RNAs]. A necessary step prior to reverse transcription was the homopolymeric (poly A) tailing of those RNAs. The rotavirus cDNA copies obtained by reverse transcription were subsequently tailed with oligo (dC) and hybridized to dG-tailed, Pst-1-digested, pBR322 plasmids. Recombinant plasmids were then used to transform competent *E. coli*. Clones carrying rotavirus cDNA were detected by colony hybridization utilizing RNA probes produced by "*in vitro*" transcription. The gene origin of the cDNA clones was determined by means of dot hybridization.

Clones representing copies of most genes of the three strains mentioned have been identified. Those representing copies of the sixth, seventh, eighth, and ninth genes (approximately 400 clones) have been characterized as to size. Those that may represent complete copies have been analyzed by restriction nuclease digestion. Forty six clones were found that specifically hybridized to the 9th gene of the Wa virus. Twenty-one of these clones had a similar size of about 1,250 base pairs. This is more than one hundred base pairs larger than the gene itself. Each of these clones exhibited the same restriction pattern. These findings suggest that these clones represent full size copies of the gene that codes for the Wa neutralization protein. Eight restriction enzymes have been identified that cut the Wa gene 9 cDNA; this knowledge should facilitate sequence analysis leading to identification of other restriction sites close to the ends of the gene which may allow the insertion of such cDNA into other vectors. Sequencing of cloned rotavirus genes is now in progress (Flores, Keith, Kalica).

Honors and Awards

Robert M. Chanock

Organizer and Co-chairman of Cold Spring Harbor Symposium, "Modern Approaches to Vaccines," Cold Spring Harbor, New York, August 31-September 4, 1983.

Invited to participate in Beecham Colloquium on Influenza commemorating the 50th anniversary of research on the influenza virus, to be held in London, England, September 20-24, 1983.

Albert Z. Kapikian

May 26-28. Invited to make presentation at "Symposium on Biological, Genetic and Immunologic Aspects of Enteropathogens in Mexico City, Mexico. Presentation on May 27, 1982 on "Prospects for Development of Rotavirus Vaccine.

July 19-21, 1982. Invited to participate in Working Conference on Rabies, Arbovirus including Dengue, Korean Hemorrhagic Fever and Viral Gastroenteritis of the Japan-U.S. Cooperative Medical Science Program. Tokyo, Japan.

July 19, 1982. Presentation at above conference on "Studies of Human Rotavirus in Adult Volunteers: induction of illness and correlates of resistance".

July 19, 1982. Cochairman of session on Viral Gastroenteritis at above meeting.

Member United States Panel on Viral Diseases of U.S.-Japan Cooperative Medical Science Program.

Aug. 10-12, 1982. Invited to participate in the 5th meeting of the Steering Committee of the Scientific Working Group on Viral Diarrheas of the Diarrheal Diseases Control Program of the WHO in Geneva. Dr. Kapikian is a member of the Steering Committee.

Sept. 6-8, 1982. Invited to attend Symposium on Enteric Infections in Man and Animals: Standardization of Immunological Procedures in Dublin, Ireland. Made presentation on Studies in Volunteers with Human Rotaviruses.

Jan. 5-7, 1983. Invited to participate in the 6th Meeting of the Steering Committee of the Scientific Working Group on Viral Diarrheas of the Diarrheal Diseases Control Program of the WHO in Geneva. Dr. Kapikian is a member of the Steering Committee.

February 9, 1983. Invited to lecture at USUHS graduate course on Animal Virology on Diarrhea Viruses.

March 1, 1983. Invited to speak with "Voice of America" Group with Dr. Flores, on Infant Diarrhea at NIH.

Invited to speak at NIAID Workshop "Search for Etiological Agents in Acquired Immune Deficiency Syndrome," April 5-6, 1983. Presentation on "Enteric

Viruses: Diagnosis by Immune Electron Microscopy," on April 6, 1983 at NIH.

Distinguished Service Medal. May 26, 1983.

Robert H. Purcell

Invited speaker, Mini Symposium on Virus B Hepatitis, George Washington University, Washington, D.C., September 10, 1982.

Invited speaker, 2nd International Symposium on Viral Hepatitis, Munich, Germany, October 19-21, 1982.

Invited speaker, Annual Meeting of the American Association for the Study of Liver Diseases, Chicago, Ill., November 2-3, 1982.

Invited speaker, Annual Meeting, American Association of Zoo Veterinarians, New Orleans, La., November 7-11, 1982.

Invited speaker, Annual Meeting of the American Association of Blood Banks, Anaheim, Calif., November 6-11, 1982.

Invited speaker, 2nd International IABS Symposium on Viral Hepatitis, Athens, Greece, November 15-18, 1982.

Invited speaker, Seminar on Hepatitis Viruses, Washington Hospital Center, Washington, D.C., December 14, 1982.

Invited speaker, Virology Seminars, Uniformed Services University of the Health Sciences, Bethesda, Md., February 7, 1983.

Invited speaker, Seminars in Virology, Johns Hopkins School of Public Health, Baltimore Md., February 11, 1983.

Panel member and speaker, U.S.-Japan Hepatitis Panel Meeting, Kyoto, Japan, March 22-23, 1983.

Organizer and speaker, Workshop on Viral Hepatitis, Poona, India, March 13-18, 1983.

Invited speaker, Workshop on AIDS, NIAID, Bethesda, MD., April 5-6, 1983.

Invited speaker, Virology Lectures, NIH, Bethesda, MD., May 9, 1983.

Invited speaker, Office of Biologics Seminar on Hepatitis Vaccines, FDA, Bethesda, Md., May 16, 1983.

Invited speaker, International Symposium on Viral Hepatitis and the Delta Agent, Turin, Italy, June 10-11, 1983.

Invited speaker, 8th International Congress of Cytology, Montreal, Quebec, Canada, June 19-23, 1983.

Richard G. Wyatt

Chairman, NIAID Animal Care Committee.

Consultant on U.S.-Egypt PL480 Project, "Etiology of Viral and Nonviral Diarrheas (03-44-C)."

Participant in Informal Consultation on AIDS in the USA and the Caribbean, NIH, April 12-13, 1983. Presented "Current US Research Effort to Isolate AIDS Agent and Diagnose AIDS Syndrome."

Co-organizer of NIAID Workshop on the search for potential etiological agents of AIDS, April 5-6, 1983, NIH.

Coordinator of NIAID Scientific Working Group on Etiology, Diagnosis, and Treatment of AIDS.

Ching-Juh Lai

Invited speaker, The International Workshop on "The Origin of Pandemic Influenza Viruses," Peking, China, November 10-12, 1982.

Participant, The EMBO Workshop on "Towards New Vaccines Against Viral Infections," Helsinki, Finland, June 21-23, 1983.

Lewis Markoff

Invited speaker, Gordon Research Conference on Animal Cells and Viruses, June 1983.

Brian R. Murphy

Invited speaker, Cold Spring Harbor Symposium, "Modern Approaches to Vaccines," August 31-September 5, 1983.

Invited speaker, Negative Strand Viruses Conference, Hilton Head, S.C., September 9-16, 1983.

Invited speaker, Beecham Colloquium, "The Molecular Virology and Epidemiology of Influenza," National Institutes for Biological Standards and Control, Hampstead, London, September 20-33.

Yasutaka Hoshino

Invited speaker, 63rd Annual Meeting of the Conference of Research Workers in Animal Diseases, Chicago, Illinois, November 8-9, 1982.

Invited speaker, The Second Annual Meeting of American Society for Virology, East Lansing, Michigan, July 10-14, 1983.

Ralston Purina Small Animal Research Award, St. Louis, Missouri, June 19, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00308-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
IN VITRO STUDIES OF HEPATITIS A VIRUS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Richard J. Daemer, Ph.D. Microbiologist LID NIAID

COOPERATING UNITS (List any)

Smith-Kline-RIT (Dr. E. D'hondt)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hepatitis A virus has been successfully adapted to growth in African green monkey kidney tissue culture. Over 28 serial passages have been achieved, and infectivity titers as high as 10^8 infectious units per ml of cell concentrate have been achieved. The virus is predominantly cell-associated and does not produce cytopathic effects (CPE). It was attenuated for chimpanzees after 10 serial tissue culture passages; reevaluation of the virus in chimpanzees after 20 tissue culture passages indicates the virus infectivity and attenuation have remained the same as at passage level 10.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00309-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

THE BIOLOGY OF HEPATITIS A VIRUS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Stephen M. Feinstone, M.D., Medical Officer, HVS, LID NIAID

COOPERATING UNITS (if any)

Fairfield Hospital, Melbourne, Australia (Dr. I. Gust); University of North Carolina (Dr. M. Sobsey)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:

1.0

PROFESSIONAL:

0.4

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have successfully isolated a strain of hepatitis A virus in African green monkey kidney tissue culture, a cell substrate suitable for vaccine development. Growth of the agent in vitro has been characterized and attenuation for chimpanzees documented. The strain of virus appears to have considerable potential for vaccine development.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00321-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

MOLECULAR VIROLOGY OF HEPATITIS A VIRUS (HAV)

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

John Ticehurst, M.D., Medical Officer, Hepatitis Viruses Section, LID NIAID

COOPERATING UNITS (if any)

Columbia University (Dr. V. Racaniello), Massachusetts Institute of Technology
(Dr. D. Baltimore)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
1.6 0.6 1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 - (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Double-stranded cDNA fragments were synthesized from hepatitis A virus (HAV) RNA and inserted into the Pst I site of pBR322. The identity of cloned cDNA was established by demonstrating its hybridization to RNA from HAV-infected tissue culture cells but not to RNA from uninfected cells. Genomic length RNA of approximately 7500 nucleotides was the predominant species that hybridized with the HAV cloned DNA. Restriction endonuclease digestion and hybridization between subgenomic fragments yielded a map of overlapping cloned cDNAs which includes 99% of the viral genome. A partial sequence from the 3' end of the genome contained 890 bases in an open reading frame preceding stop codons, 60 bases of a noncoding region, and a tract of poly(A).

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00310-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

HYBRIDOMA ANTIBODIES TO PATHOGENIC VIRUSES

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Stephen M. Feinstone, M.D., Medical Officer, HVS LID NIAID

COOPERATING UNITS (if any)

NIAID, NIH, Bethesda, Maryland (Dr. A. Fauci)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:

1.6

PROFESSIONAL:

1.6

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Attempts to produce monoclonal antibody to hepatitis A virus are in progress. The development of new serologic tests for detection of monoclonal anti-HAV will simplify the procedure. Two specific monoclonal antibodies have been produced and are being analyzed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00311-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

SEARCH FOR NEW HEPATITIS AGENTS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Robert H. Purcell, M.D. Head, Hepatitis Viruses Section LID NIAID

COOPERATING UNITS (if any)

Clinical Center Blood Bank (Dr. H. Alter); National Institute of Virology, Pune, India (Dr. K. Pavri); Medical College of Srinagar, India (Dr. M. S. Khuroo); Mt. Sinai Hospital (Dr. H. Popper).

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:

1.0

0.2

0.8

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

New hepatitis agents continue to be recognized. Recently, a form of epidemic hepatitis occurring in India was found not to be caused by recognized hepatitis viruses. Attempts to transmit an agent from acute-phase clinical samples to primates are in progress. Attempts to identify and characterize a new agent, through collaborative studies, are also in progress. A workshop on hepatitis A virus was planned and held at the National Institute of Virology, Pune, India.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00312-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

CLINICAL AND EXPERIMENTAL STUDIES OF HEPATITIS B VACCINES

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

John Ticehurst, M.D., Medical Officer, Hepatitis Viruses Section LID NIAID

COOPERATING UNITS (if any)

Baylor School of Medicine (Dr. B. Hollinger); Scripps Institute (Dr. R. Lerner), Div. of Molecular Virology and Immunology, Georgetown U., Washington, D. C. (Dr. J. Gerin), LBV (Dr. B. Moss).

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LABORATORY OF INFECTIOUS DISEASES

SECTION

HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

0.3

0.3

0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical testing and characterization of plasma-derived hepatitis B vaccine prepared by the NIAID will soon be completed. The vaccine is highly immunogenic, safe and well tolerated. An efficacy trial of the vaccine in infants is in progress in Asia. Second generation hepatitis B vaccines are currently under development. Approaches include the use of recombinant DNA-derived viral antigen and synthetic peptides. Preliminary results of the former are encouraging; preliminary results with the latter suggest that problems associated with immunogenicity must be overcome before synthetic peptide vaccines for hepatitis B will be practical.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00313-02 LID
PERIOD COVERED <u>October 1, 1982 through September 30, 1983</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>MOLECULAR BIOLOGY OF HEPATOCELLULAR CARCINOMA</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <u>Richard J. Daemer, Ph.D. Microbiologist LID NIAID</u>		
COOPERATING UNITS (if any) <u>Office of Biologics, Bethesda, Maryland (Dr. B. Merchant)</u>		
LAB/BRANCH <u>LABORATORY OF INFECTIOUS DISEASES</u>		
SECTION <u>HEPATITIS VIRUSES SECTION</u>		
INSTITUTE AND LOCATION <u>NIAID, NIH, BETHESDA, MARYLAND 20205</u>		
TOTAL MANYEARS: <u>0.8</u>	PROFESSIONAL: <u>.</u>	OTHER: <u>0.0</u>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>Many tumor-bearing animals develop antibodies to unique antigens associated with the oncogenic virus causing the tumor. These antigens, called "neoantigens," have been found in tumors caused by papovaviruses, adenoviruses, and herpes viruses. Hepatitis B virus, a hepadnavirus with suspected oncogenic potential, cannot be transmitted to non-primates but patients with HBV-associated hepatoma might be expected to have antibody to a HBV-associated neoantigen if one exists. Using a hepatoma cell line that contains integrated HBV DNA, we have sought immunofluorescent antibody in sera of hepatoma patients. Approximately seven percent of sera from HBsAg-positive hepatoma patients contain an antibody that reacts with a nuclear antigen in the hepatoma cell line. This antigen has been found in another hepatoma cell line that also contains integrated HBV genome but not in two other hepatoma cell lines lacking HBV genome. The antigen is being further characterized to determine if it is the product of a transforming gene.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00314-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

WOODCHUCK HEPATITIS VIRUS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Robert Purcell, M.D. Head, Hepatitis Viruses LID, NIAID

COOPERATING UNITS (if any)

Div. of Molecular Virology & Immunology, Georgetown U., Washington, D.C., (Dr. J. Gerin); New York State College of Veterinary Medicine (Dr. B. Tenant)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.4	0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The woodchuck hepatitis virus (WHV) is taxonomically and serologically related to the hepatitis B virus. Infection with each of these viruses is associated with acute and chronic hepatitis and hepatic cell carcinoma in their respective hosts; these associations appear to be etiological in nature. The WHV/woodchuck model system provides a convenient means of studying the relationship between virus and host in the oncogenic process. Tests specific for the WHV antigen-antibody systems have been developed. They are being applied to a prospective study of WHV infection in newborn woodchucks and the ability of active immunization to prevent hepatic cell carcinoma. The results of the study should have important prognostic value in evaluating active immunoprophylaxis of hepatitis B virus in man.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00315-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

SEARCH FOR NEW HEPADNAVIRUSES

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

John Ticehurst, M.D., Medical Officer, Hepatitis Viruses Section, LID NIAID

COOPERATING UNITS (if any)

Veterinary Resources Branch, NIH, (Dr. R. Whitney)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 - (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Viruses similar to hepatitis B virus ("hepadnaviruses") have been identified in three non-human species: the eastern woodchuck, the Beechey ground squirrel and the Pekin duck. It is likely that many other species harbor similar viruses. The existing animal hosts are of limited value because inbred strains suitable for detailed immunological studies are not available. We are searching for hepadnaviruses among inbred strains of rodents, especially those with a known high incidence of hepatoma, in hopes of finding a more useful animal model system. Sensitive assays of hepadnavirus infection have been modified to permit testing of the small quantities of serum available.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00316-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

THE DELTA AGENT

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Robert H. Purcell, M.D. Head, Hepatitis Viruses Section, LID NIAID

COOPERATING UNITS (if any)

Georgetown U., Washington, D.C., (Dr. J. Gerin); Centers for Disease Control, Phoenix, Ariz. (Dr. S. Hadler); Mt. Sinai Hospital, N.Y.C. (Dr. H. Popper).

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
0.6 0.5 0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The delta agent is a transmissible hepatitis agent that appears to be defective and requires co-infection with hepatitis B virus for its own synthesis. The agent was discovered in 1977 in Italy, where it is endemic. Evidence for infection with the delta agent is found most frequently in carriers of hepatitis B virus who are repeatedly exposed to blood (hemophiliacs, illicit drug users, etc.). Sensitive assays for delta agent infection have been developed and used to evaluate experimental infection of HBV-carrier chimpanzees. In both man and chimpanzee infection with the delta agent results in very severe hepatitis. The delta agent has been experimentally transmitted to woodchucks chronically infected with the woodchuck hepatitis virus, a hepatitis virus similar to hepatitis B virus. The chimpanzee and woodchuck provide animal model systems for more detailed characterization of this medically important agent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00317-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

BIOLOGY OF NON-A, NON-B HEPATITIS AGENTS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Stephen M. Feinstone, M.D., Medical Officer, HVS, LID NIAID

COOPERATING UNITS (if any)

Clinical Center Blood Bank, NIH (Dr. H. Alter and Dr. H. Shiraishi)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.3	0.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although non-A, non-B hepatitis agents cannot be detected by serologic means, they can be experimentally transmitted to chimpanzees and marmosets. These species have been useful in determining the infectivity titers of various non-A, non-B virus containing plasmas. Although most plasmas contain only 10^2 - 10^3 infectious units per ml, one plasma was found to contain over 10^6 infectious units per ml. This plasma has provided an inoculum suitable for characterization of the agent. We have recently demonstrated that at least one non-A, non-B agent contains essential lipids, a characteristic that will be important in the classification and, probably, the control of non-A, non-B hepatitis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI AI 00318-02 LID

PERIOD COVERED

October 31, 1982 through September 31, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

MOLECULAR BIOLOGY OF NON-A, NON-B HEPATITIS AGENTS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Robert H. Purcell, M.D. Head, Hepatitis Viruses Section LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.4	0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Attempts to identify non-A, non-B hepatitis agents by serologic means have been uniformly unsuccessful throughout the world. We have sought to apply recent advances in nucleic acid chemistry to a search for the genome of the non-A, non-B agent. Sensitive radiolabeling procedures have been modified to permit labeling of minute quantities of nucleic acid. Identification of the genome of the non-A, non-B agent would permit its characterization and cloning.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00319-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NEW APPROACHES TO THE IN VITRO PROPAGATION OF NON-CULTIVATABLE AGENTS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Richard J. Daemer, Ph.D. Microbiologist LID NIAID

COOPERATING UNITS (if any)

Johns Hopkins University Medical School, Baltimore, Md. (Dr. L. Widman)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There is a need for an in vitro substrate for the cultivation of hepatitis viruses. Hepatocytes would seem a logical choice, but it is very difficult to obtain and maintain primate hepatocytes in culture. We are attempting to develop hepatocyte-hepatoma hybridomas of primate origin. Such hybrid cells would be expected to have the receptor sites and metabolic systems suitable for synthesis of hepatitis viruses and the ability of hepatoma cells to multiply indefinitely in vitro.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00320-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

PARVOVIRUS-LIKE AGENTS AND HEMATOLOGICAL DISORDERS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Robert H. Purcell, M.D., Head, Hepatitis Viruses Section LID NIAID

COOPERATING UNITS (if any)

NHLBI (Dr. H. Young), Children's Hospital, Washington, D.C. (Dr. Rodriguez).

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:

0.2

0.1

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Severe hypoplastic crisis among patients with sickle cell anemia has been associated with infection with what appears to be a non-cultivable parvovirus-like agent. Immune adherence hemagglutination and radioimmunoassay techniques have been adapted to identification of this agent and its antibody: approximately 60 percent of normal populations have been found to be antibody-positive. Attempts to transmit the serum parvovirus-like agent to primates are in progress. Attempts to demonstrate replication in tissue culture have been partially successful.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00370-01 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

STUDIES OF ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS)

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Robert H. Purcell, M.D., Medical Officer LID, NIAID

COOPERATING UNITS (if any)

NINCDS (Dr. W. London); New York Blood Center (Drs. C. Stevens and L. Baker); Memorial Sloan Kettering Institute (Dr. J. Gold), Laboratory of Immunogenetics (Dr. T. Folks).

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LABORATORY OF INFECTIOUS DISEASES

SECTION

HEPATITIS VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
0.2 0.1 0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A new medical syndrome, Acquired Immune Deficiency Syndrome (AIDS) has recently been recognized. It is characterized by profound progressive depression of the immune system, resulting in repeated opportunistic infections and at least one type of neoplasm.

The syndrome is usually if not always fatal. It is epidemic in the United States among certain "high-risk populations (male homosexuals, illicit drug users, Haitians, and, to a lesser extent, hemophiliacs, recipients of blood transfusions and intimate contacts of cases). The epidemiology of the syndrome suggests that it is caused by a transmissible agent. Attempts to transmit a putative AIDS agent to chimpanzees and monkeys are in progress.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00322-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

SEQUENCE ANALYSIS OF RESPIRATORY SYNCYTIAL (RS) VIRUS NUCLEOCAPSID GENE

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

S. Venkatesan, M.D. Expert, Respiratory Viruses Section LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.7	0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recently, we obtained several cDNA clones encoding the RS viral nucleocapsid protein (NC), matrix protein (M), phosphoprotein (P) and a nonstructural protein (NS₂) (Venkatesan et al., PNAS, 1983). We have since sequenced a cDNA plasmid encoding the entire coding sequence of the NC gene. The 3' terminus of this gene has been identified by the presence of 14 poly A residues at one end of the clone. The cloned DNA lacks 6 nucleotides of the 5' end of the mRNA as determined by primer extension and gene walking on the mRNA. However, a single open reading frame of 1412 nucleotides encoding a protein of 467 amino acids is present within the cloned DNA. This translated protein of 51540 daltons is rich in basic amino acids, relatively rich in proline, but poor in cysteine. It has no sequence homology with the capsid proteins of other negative strand RNA viruses implying that RS virus is evolutionarily distinct. Interestingly, the sequence upstream of the poly A tail of this gene was not homologous to a similar region in the other RS viral genes.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00368-01 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

SEQUENCE ANALYSIS OF THE 3' END(s) OF RS VIRAL AND PARAINFLUENZA GENOME

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

S. Venkatesan, M.D. Expert, Respiratory Viruses Section LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MD 20205

TOTAL MANYEARS:

0.5

PROFESSIONAL:

0.2

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

RS virus despite its poor growth in vitro, has been grown to high titer by us and this has allowed us to purify virus by successive cycles of sucrose gradient centrifugation. In addition to genomic 50S RNA, purified virus preparations contain cellular RNA contaminants. The 50S genomic RNA was purified away from these contaminants and 3' end labeled by standard procedures. The 50S RNA ³²P labeled at the 3' end only hybridized to RS virus cDNA clones and not to cellular cDNA clones. By independent hybridization studies the identity of 50S RNA as genomic RS virus RNA was established. Similarly, RS viral nucleocapsids were processed and found to contain substantial amounts of both negative and positive genomes. Studies with parainfluenza 3 virus along such lines yielded similar results.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00323-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DIRECT EXPRESSION OF PARAMYXOVIRUS PROTEINS IN EUKARYOTIC CELLS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

S. Venkatesan, M.D. Expert, Respiratory Viruses Section LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Md. 20205

TOTAL MANYEARS:

0.3

PROFESSIONAL:

0.2

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Alternate shuttle vector plasmid systems capable of replicating both in bacteria and animal cells could provide immediate instant expression of cDNAs cloned in such cells. By inserting cDNA within the appropriate region, it is possible to express exogenous genes in monkey cells stably transformed by SV40 (cos cells); these cells are permissive for replication of recombinant SV40-plasmids.

We are in the process of constructing a cDNA library in the above system using as templates, mRNA from cells infected with RS virus or parainfluenza 3 virus. Cells were treated with high doses of actinomycin D during infection to reduce the level of cell specific mRNAs. These recombinant plasmids in groups will be used to transfet monkey (cos) cells and the in vitro translational products will be identified by a variety of immunological methods.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00344-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

IMMUNOLOGIC CHARACTERIZATION OF RESPIRATORY SYNCYTIAL VIRUSES

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Gregory Prince, D.D.S., Ph.D.

Expert, RSV Section

LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:

0.8

PROFESSIONAL:

0.3

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Earlier efforts to develop a vaccine for RS virus were directed primarily at genetic alteration of wild-type human RS virus. To date none of these efforts has proven successful. Recently an avian-adapted strain of human RS virus (HRSV), thought to be attenuated, was tested in chimpanzees; unfortunately, it was observed to be fully virulent. Previous investigations of bovine RS virus (BRSV) were extended, and six strains have now been evaluated in cotton rats. Several show promise in protecting animals from HRSV infection. Further evaluation in primates is contingent upon development of a technique for sampling the lower respiratory tract for RS virus by pulmonary lavage.

Two species of monoclonal antibody have been tested in cotton rats for effectiveness in protecting against HRSV infection. As more antibody species are obtained, we plan to determine the role of each species in immunity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00345-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

IMMUNITY TO RS VIRUS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Gregory Prince, D.D.S., Ph.D.

Expert, RSV Section

LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: PROFESSIONAL:

0.8

0.3

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previously we observed that resistance to infection of the lungs by respiratory syncytial virus (RSV) was transferred from immune cotton rats to their non-immune parabiotic partners. Passive transfer experiments suggested that serum, rather than cells, mediated this protective effect, as cotton rats pretreated with either cotton rat or human serum containing RS virus antibodies were completely resistant to pulmonary infection. Duplication of this effect using purified human IgG identified serum antibody as the effector. Human antiserum (or purified IgG) administered to cotton rats after RSV infection resulted in complete or near-complete clearance of pulmonary virus, suggesting a potential technique for specific treatment of established RSV disease.

Potentiated disease that developed during RS virus infection of individuals previously given a formalin-killed RSV vaccine (1966-1967) remains unexplained. Using the cotton rat we have developed an experimental model for examining vaccine-induced potentiation. Early results suggest an Arthus-type reaction.

RSV was inoculated into a second strain of cotton rats, in which it produced overt signs of disease for the first time. Efforts are under way to establish a colony of this strain of cotton rat.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00367-01 LID

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

NONSTRUCTURAL PROTEIN(S) OF RESPIRATORY SYNCYTIAL VIRUS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

S. Venkatesan, M.D. Expert, RSV Section LID NIAID

COOPERATING UNITS (if any)

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LABORATORY OF INFECTIOUS DISEASES

SECTION

RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.7	0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Respiratory syncytial (RS) virus has been reported to encode 2 or 3 nonstructural proteins. At least one of these, henceforth referred to as NS₂, was shown earlier to be encoded by a distinct transcript. This finding is based on the identification of several independent cDNA clones that hybrid selected mRNA that was translated *in vitro* to yield NS₂ as its sole translation product. A cDNA insert from the largest recombinant plasmid encoding this gene has been sequenced. The cDNA insert has approximately 1050 bp of RS viral sequence and is about 350 bases larger than the mRNA encoding this protein as determined by Northern blot analysis. Analysis of the DNA sequence revealed two different open reading frames, encoding polypeptides with 137 or 123 amino acids. In one reading frame, translation starts about 50 nucleotides from 5' end of the messenger strand and stops 400 nucleotides downstream. In this reading frame there is an untranslated sequence of about 580 nucleotides between the stop codon and the poly (A) tail. In the other reading frame translation starts about 570 nucleotides from the 5' end and ends 387 nucleotides downstream, 90 nucleotides short of the poly (A) tail.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00371-01 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

RS VIRUS MATRIX AND PHOSPHOPROTEIN GENES

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

S. Venkatesan, M.D. Expert, RSV Section

LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.7	0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Amino acid sequence of RS virus matrix protein was deduced from sequencing a recombinant plasmid containing a cDNA copy of this gene. The cDNA clone lacks about 10 nucleotides of the 5' end of the mRNA but the 3' end is preserved. There is one long open reading frame encoding 250 amino acids that presumably constitutes the matrix protein. There is also another open reading frame which overlaps with the long open reading frame. This second open reading frame encodes a protein of 75 amino acids. Whether the matrix protein gene actually produces an additional viral protein is being investigated. It should be noted that certain recombinant plasmids containing the matrix protein gene hybrid select viral mRNA(s) that yield upon translation the matrix protein and another protein that comigrates with a viral nonstructural protein (NS₂). However, only an mRNA 1000 bases long hybridizes with this plasmid.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00372-01 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

RECOMBINANT cDNA PLASMIDS CONTAINING RS VIRUS SURFACE GLYCOPROTEINS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

S. Venkatesan, M.D. Expert Respiratory Viruses Section LID NIAID

COOPERATING UNITS (if any)

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LABORATORY OF INFECTIOUS DISEASES

SECTION

RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MD. 20205

TOTAL MANYEARS:

0.8

PROFESSIONAL:

0.5

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recently, we constructed a cDNA library in *E. coli* using mRNAs from RS virus-infected cells. By a process of hybrid selection of viral mRNAs using recombinant cDNA plasmids and subsequent cell-free translation of the selected mRNAs, recombinants encoding the viral nucleocapsid protein, phosphoprotein, matrix protein and a nonstructural protein were identified. This kind of analysis failed to identify the viral glycoprotein genes since putative precursors for these proteins were not translated *in vitro* from viral mRNA(s).

By a process of colony hybridization, we have grouped several recombinant plasmids from our cDNA library that represent RS viral genes distinct from those previously identified. Successive Northern blot analysis of mRNA from infected cells and uninfected cells allowed us to identify two classes of recombinants, one hybridizing with an RNA 2200 nucleotides in length and another with an RNA 1000 nucleotides long.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00324-02 LID

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

LABORATORY STUDIES OF MYXOVIRUSES

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Brian R. Murphy, M.D. Medical Officer LID NIAID

COOPERATING UNITS (if any)

St. Jude Children's Hospital, Memphis, Tenn.; Cornell Univ. School of Vet. Medicine, Ithaca, N.Y.; Scripps Institute, La Jolla, California

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:

4.2

PROFESSIONAL:

2.2

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Influenza A reassortant viruses that contain human influenza hemagglutinin and neuraminidase genes and the six internal genes from the A/Mallard/78 (H2N2) avian influenza donor virus were readily generated in vitro and were attenuated for monkeys and man. An avian-human influenza reassortant containing only an M gene or NP gene from the avian virus was attenuated for monkeys. The internal six genes of two additional avian virus also effected restriction of viral replication in primates. The avian hemagglutinin and neuraminidase genes are required for enterotropism in ducks. The H2 hemagglutinin gene specifies restricted replication in nasal turbinates of hamsters. Synthetic peptides representing a wide range of regions on the H3₇₅ hemagglutinin stimulate antibodies that bind to the hemagglutinin; however, immunization of hamsters with these peptides did not induce resistance to challenge.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00325-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

STUDY OF RESPIRATORY VIRUSES IN PRIMATES

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Brian R. Murphy, M.D. Medical Officer LID NIAID

COOPERATING UNITS (if any)

MeIoy Laboratories, Rockville, Maryland; NINCDS, NIH

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LABORATORY OF INFECTIOUS DISEASES

SECTION

RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:

1.3

PROFESSIONAL:

0.3

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An influenza A virus isolated from seals replicated in the trachea and nasopharynx of squirrel monkeys to a level similar to virulent human virus. This virus initiated a systemic infection involving the spleen, liver, and muscle.

Transfer of the six internal genes of the avian A/Mallard/78 (H2N2) virus (i.e., genes that code for nonsurface viral proteins) to three human influenza viruses (H1N1 or H3N2) reproducibly attenuated the resulting reassortant viruses for monkeys and hamsters. When tested in monkeys the human A/Wash/80 X avian A/Mall/78 reassortant was satisfactorily attenuated, not transmissible, stable as regards phenotype, and immunogenic. Immunogenicity was demonstrated by the induction of resistance to challenge with wild-type virulent human virus. These studies form the basis for evaluation of these promising reassortants in man. The NP and M genes of the avian A/Mallard/78 (H2N2) virus donor appear to play the greatest role in conferring attenuation on avian-human influenza virus reassortants. The 6 internal genes of 2 other avian influenza viruses [A/Mallard/Alberta/78 (H1N1) and A/Pintail/Alberta/79 (H7N8)] that grow poorly in monkeys confer this restriction upon human-avian influenza virus reassortants into which they are transferred. With each of 3 avian influenza viruses tested thus far one or more internal genes constitute the basis for restriction of replication in primate respiratory tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00326-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

STUDY OF RESPIRATORY VIRUSES IN VOLUNTEERS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Brian R. Murphy, M.D. Medical Officer LID NIAID

COOPERATING UNITS (if any)

Flow Labs., Rockville, Md.; U. of Md. Sch. Med., Balto., Md., U. Rochester, Sch. of Med., Rochester, N.Y.; NCI, Immunology Br., Bethesda, MD.; U. Vermont, Burlington, Vt.; Vanderbilt U., Nash. TN; BOB, FDA, Bethesda, MD.

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LABORATORY OF INFECTIOUS DISEASES

SECTION

RESPIRATORY VIRUSES SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:

3.5+

PROFESSIONAL:

0.5

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The human infectious dose₅₀ (HID₅₀) of current H1N1 and H3N2 cold-adapted (ca) reassortant viruses ranges from $10^{5.5}$ to $10^{6.9}$ TCID₅₀. The six internal genes (i.e., genes that code for nonsurface viral proteins) of the ca donor virus reproducibly confer on wild-type viruses (1) satisfactory attenuation, (2) immunogenicity, (3) phenotypic stability, and (4) non-transmissibility. Infection with 70 HID₅₀ of a ca reassortant induced greater resistance to wild-type virus challenge than did inactivated influenza vaccine.

The safety, immunogenicity, and HID₅₀ of a human-avian influenza reassortant containing the 6 internal genes of its avian influenza virus parent was determined in seronegative volunteers. The reassortant was satisfactorily attenuated. Vaccinees shed significantly less virus than individuals infected with wild-type virus. Preliminary analysis indicates that the immunogenicity of this virus is similar to that of the cold-adapted reassortants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00327-02 LID
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) CHARACTERIZATION OF RESPIRATORY VIRUSES USING MONOCLONAL ANTIBODIES		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Kathleen L. van Wyke, Ph.D. Senior Staff Fellow, RV Section LID NIAID		
COOPERATING UNITS (if any) University of Alabama, Birmingham, AL; Wistar Institute, Philadelphia, PA; St. Jude Children's Research Hospital, Memphis, TN		
LAB/BRANCH LABORATORY OF INFECTIOUS DISEASES		
SECTION RESPIRATORY VIRUSES SECTION		
INSTITUTE AND LOCATION NIAID, NIH, BETHESDA, MARYLAND 20205		
TOTAL MANYEARS:	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>Five monoclonal antibodies to the matrix protein of the influenza A virus have defined three antigenic sites. Two of the three undergo genetic variation of a type illustrative of genetic dimorphism also seen with surface antigens. Monoclonal antibodies to the four epitopes of the A/Udorn/72 hemagglutinin were produced that recognized both glycosolated and unglycosolated hemagglutinins produced in tissue cultures infected with a SV40-HA recombinant. IgA monoclonal antibodies have been produced to influenza A virus proteins in an effort to characterize the local antibody response to viral pathogens. Monoclonal antibodies to paramyxovirus and respiratory syncytial virus are being produced to characterize the antigenic composition of the virus proteins.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00328-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DEFECTS OF INFLUENZA HEMAGGLUTININS ALTERED AT THE HYDROPHOBIC CARBOXY-TERMINUS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Michael M. Sveda, Ph.D. Expert, MBV Section LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

MOLECULAR VIRAL BIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.7	0.3

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Requirements for cell surface expression of the influenza hemagglutinin (HA) were studied using a recombinant of SV40 which had full-length DNA sequences coding for the influenza virion HA inserted into the late region of SV40 DNA and propagated in the presence of tsA SV40 helper. Infection of primate cells with the SV40-HA recombinant produced a functional glycosylated HA polypeptide that accumulated on the cell surface. To delineate the protein domains necessary for surface expression of the HA polypeptide, mutations were engineered in the recombinant SV40-HA DNA. One mutant of interest sustained a deletion of 5 base pairs at the Bam HI site generating a shift in reading frame for the hydrophobic sequence of 24 amino acids as well as the C' terminal 13 amino acids. This shift yielded a terminal sequence in which 21 of 24 amino acids were hydrophobic or neutral followed by 4 charged amino acids. Although its C' terminus resembled wild-type topologically, the mutant HA was not secreted, was not expressed on the cell surface, and was only present intracellularly. These findings suggest a role for the hydrophobic COOH-terminus not only in cell surface expression but in secretion related glycosylation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00329-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

EXPRESSION OF THE CLONED DNA OF INFLUENZA A VIRUS NEURAMINIDASE

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Lewis Markoff, M.D.

Medical Officer

LID NIAID

COOPERATING UNITS (if any)

St. Jude Childrens' Hospital, Memphis, Tennessee (Dr. Robert Webster)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

MOLECULAR VIRAL BIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:

1.1

PROFESSIONAL:

0.8

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A full-length ds DNA copy of an influenza A N2 neuraminidase (NA) gene was cloned into the late region of SV40 in a hybrid expression vector that includes pBR322 plasmid DNA sequences and the SV40 early region and SV40 late region mRNA intervening sequences thought to stabilize late mRNA transcripts. The cloned wild-type NA was shown to be present in the cytoplasm of fixed cells and at the surface of "live" (unfixed) cells by indirect immunofluorescence using N2 monoclonal antibodies. Immunoprecipitation of ³⁵S-methionine labeled proteins from recombinant cells using heterogeneous N2 antibody showed that the product of the cloned NA DNA comigrated with glycosylated NA from influenza virus infected cells, remained associated with the membrane fraction, and could form an immunoprecipitable dimer. Using a low molecular weight substrate for the NA that releases a fluorescing moiety upon hydrolysis, NA enzymatic activity was detectable after SV40 lysis of vector-infected cells. These properties of the product of the cloned wild-type gene were compared to those of the polypeptides produced by three deletion mutant NA DNAs that were also cloned into the late region of the SV40 vector. These mutants lacked 7 (dlk), 21 (dliI) or all 23 amino acids (dlz) of the amino-(N-) terminal variable hydrophobic region thought to anchor the mature wild-type NA tetrameric structure in the infected cell or influenza viral membrane. Comparison of the phenotypes of these mutants showed that this region in the NA molecule functions not only in membrane anchorage but also as a signal sequence, permitting entry of the nascent NA polypeptide into membrane organelles for glycosylation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00330-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

EXPRESSION OF INFLUENZA VIRUS NUCLEOPROTEIN USING AN SV40 VECTOR

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Bor-Chian Lin, Ph.D. Visiting Associate, MVB Section LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

MOLECULAR VIRAL BIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An influenza A virus nucleoprotein (NP) gene was cloned from DNA sequences derived by reverse-transcription of virion RNA segments using synthetic oligonucleotide primers as detailed previously. The cloned NP DNA coding for a full-length copy of a virion NP RNA segment was verified by the size and the completeness of DNA sequences at both terminal ends. The influenza A virus nucleoprotein (NP) gene was cloned into the BamH1 site of the late region of SV40 in an SV40-pBR322 expression vector. African green monkey primary cells transfected with the SV40-NP recombinant DNA in the presence of an early SV40 ts mutant helper, synthesized a polypeptide that was specifically immunoprecipitable with NP monoclonal antibodies and that had a molecular weight of 56 K daltons identical to the NP of influenza virus as estimated on SDS-polyacrylamide gels. The putative NP was detected in the nucleus of infected primate cells by an indirect immunofluorescence assay. This nuclear localization of NP from recombinant DNA was similar to that seen during influenza virus infection suggesting the NP product may be functionally active.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00369-01 LID
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) PERSISTANT EXPRESSION OF INFLUENZA VIRUS NUCLEOPROTEIN FROM CLONED DNA		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Ching-Juh Lai Head, MBV Section LID NIAID		
COOPERATING UNITS (if any) Ming-fan Law, Ph.D., Expert, LCP, NCI Peter Howley, M.D., LCP, NCI		
LAB/BRANCH LABORATORY OF INFECTIOUS DISEASES		
SECTION MOLECULAR VIRAL BIOLOGY SECTION		
INSTITUTE AND LOCATION NIAID, NIH, BETHESDA, MD. 20205		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Selective complementation of defective influenza A virus strains and ultimately "rescue" of cloned mutant influenza DNA into influenza virions may require the expression of cloned genes in persistently infected or stably transformed cells. For example, one obstacle to achieving complementation and rescue during lytic infection by our established SV40 vectors is interference of co-infecting influenza virus by replicating SV40. Bovine papilloma virus (BPV) is a large DNA virus that replicates autonomously and extra-chromosomally during persistent infection of animal cells. We recently initiated a collaborative effort with Drs. Peter Howley and Ming-fan Law (NCI) in an effort to exploit their BPV vector for the expression of cloned influenza viral genes. We constructed a BPV recombinant DNA incorporating the influenza nucleoprotein (NP) gene and transformed a mouse cell line (C127) for the persistent expression of NP.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00331-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

TRANSCRIPTION OF INFLUENZA A VIRUS: SYNTHESIS OF SPLICED AND UNSPLICED mRNAs

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Ching-Juh Lai, Ph.D. Head, Molecular Viral Biology Section LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

MOLECULAR VIRAL BIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:

0.7

PROFESSIONAL:

0.2

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Following infection of a permissive cell, influenza viral cytoplasmic mRNA's are transcribed from the virion RNA segments. We showed previously that both uninterrupted (colinear) and interrupted mRNA species are derived from viral RNA segments coding for the matrix protein (M) and non-structural proteins (NS). To demonstrate that interrupted mRNA's are generated by splicing, cloned cDNA was inserted into an SV40 vector so that the late SV40 transcription signals directed the synthesis of (+) strand NS RNA sequences. Poly A-containing RNA from African green monkey kidney cells infected with the SV40-NS recombinant was analyzed for the presence of NS sequences. A colinear and an interrupted NS RNA species were produced. The interrupted NS RNA contained a splice sequence at the juncture similar to that of NS₁ mRNA found during influenza infection. We examined the NS polypeptides produced by the SV40-NS recombinant using immunoprecipitation and gel electrophoresis. As predicted, the the NS₁ and NS₂ of influenza virus were detected. Specific splicing from the transcript of SV40-NS DNA ruled out the possibility that influenza mRNA's are formed by polymerase "jumping" at the consensus sequences during replication. The presence of colinear as well as spliced NS mRNA's establishes that processing occurs during in vivo influenza virus transcription.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00332-02 LID
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) ALLEL E REPLACEMENT OF INFLUENZA VIRUS GENE SEGMENTS USING CLONED DNA SEQUENCES		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Ching-Juh Lai, Ph.D. Head, MVB Section LID NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH LABORATORY OF INFECTIOUS DISEASES		
SECTION MOLECULAR VIRAL BIOLOGY SECTION		
INSTITUTE AND LOCATION NIAID, BETHESDA, MARYLAND 20205		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects	<input type="checkbox"/> (b) Human tissues	<input checked="" type="checkbox"/> (c) Neither
<input type="checkbox"/> (a1) Minors		
<input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>We obtained cloned full-length DNA copies of influenza viral gene segments that code for the nonstructural proteins (NS), membrane protein (M), neuraminidase (NA), nucleoprotein (NP), hemagglutinin (HA), and polymerase protein (P3). Cloned complete DNA sequences were inserted into the late region of SV40 and the recombinant DNA was propagated in the presence of a tsA SV40 helper. SV40-influenza DNA recombinants that synthesize either the (+) or the (-) full-length strand of influenza viral transcripts were isolated for use in allele replacement, that is, conversion of cloned DNA back into virion RNA. As a first step, we employed the technique of "marker rescue" under antibody selection using the H1N1 subtype of an influenza virus (strain WSN) and an SV40-HA (H3) or SV40-NA (N2) recombinant that produced the (+) strand of influenza viral RNA transcripts in a coinfecting cell. Similarly, "marker rescue" of an influenza <u>ts</u> mutant defective in P3 was tested with an SV40-P3 recombinant that transcribed the (+) strand of influenza viral P3 RNA. Preliminary characterization by immunologic techniques and/or gel analysis did not identify progeny that contained a substituted allele corresponding to a rescued viral gene and seven remaining genes from WSN virus. Experiments using recombinants that transcribe the (-) strand viral RNA are under way to determine whether this approach will prove successful for use in allele replacement.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00365-01 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DEFECTS OF AN INFLUENZA VIRUS HEMAGGLUTININ LACKING THE SIGNAL SEQUENCES

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Ching-Juh Lai, Ph.D. Head, Molecular Biology Section, LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

MOLECULAR VIRAL BIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MD 20205

TOTAL MANYEARS:

2.3

PROFESSIONAL:

2.1

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Wild type HA-SV40 recombinant cloned at the unique Bam H1 site of pBR322 was used for the derivation of HA deletion mutants. A series of deletion mutations were introduced at a specific MboII site located 12 base pairs downstream of the initiation codon of HA. We identified one mutant with an in-phase deletion. The in-phase mutant HA sustained a deletion of 33 bp or 11 amino acids, all within the signal peptide. Sequence analysis predicted that 5 amino acids of the 16 amino-acid signal sequence remained as a result of this deletion. The signal peptide cleavage site of Gly-Gln and the subsequent downstream sequences were not affected. Mutant HA lacking the signal sequences accumulates in the cytoplasm but is not incorporated into the outer membrane. The mutant polypeptide synthesized during infection was not modified by glycosylation. This failure to agglutinate red blood cells suggests that the mutant HA remains monomeric with a resulting defect in forming trimeric structures which are required for functional activity. Alternatively, the functional defect of mutant HA may be due to the lack of carbohydrate components and as a result the mutant HA subunit fails to assemble properly.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT,

PROJECT NUMBER

ZO1 AI 00366-01 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

MOLECULAR BIOLOGY OF DENGUE VIRUSES

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

C.-J. Lai Head, Molecular Viral Biology Section, LID NIAID

COOPERATING UNITS (if any)

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LABORATORY OF INFECTIOUS DISEASES

SECTION

MOLECULAR VIRAL BIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Dengue viruses belong to the flavivirus group whose members also include yellow fever, Japanese B encephalitis, Russian spring-summer encephalitis, and other less characterized but highly pathogenic viruses. Dengue infection is usually manifested by fever, severe debility and bone pain; however, mortality is rare except in the dengue hemorrhagic fever-shock syndrome that occurs in children. A successful immunization procedure for prevention of dengue infection has not been developed.

Dengue virus type 4, the most recent isolate associated with epidemics in the Western hemisphere, was chosen to initiate our molecular biology study. Virion RNA extracted from virus preparations was analyzed by electrophoresis on formaldehyde-agarose gels. A majority of vRNA appeared to be full-length migrating as a single band. To reverse-transcribe the dengue vRNA, we first carried out polyadenylation at the 3'-terminus using the E. coli poly A addition enzyme. The poly A-dengue RNA will be used for reverse-transcription using oligo (dT) as a primer. The single-stranded complementary DNA transcripts will be converted to DNA duplexes for cloning in E. coli.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00343-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

THE ROLE OF NORWALK-LIKE 27 nm VIRUS PARTICLES IN VIRAL GASTROENTERITIS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Harry Greenberg, M.D. Medical Officer LID NIAID

COOPERATING UNITS (if any)

Calif. State Health Dept., Berkeley, Calif., (L. Oshiro); Dept. of Medicine, Michigan State U. (M. Gurwith); Bureau of Epidemiology, Washington, D. C. and Atlanta, Ga. W. Gary, R. Baron)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A variety of 27 to 32 nm viruses have been shown to be etiologically associated with epidemic nonbacterial gastroenteritis. The Norwalk virus is the most thoroughly studied member of this group. Seroepidemiologic studies using a sensitive solid phase RIA indicate: (1) Norwalk virus has a world wide distribution, (2) Norwalk infection is responsible for at least 1/3rd of outbreaks of nonbacterial gastroenteritis in the U.S., and (3) the majority of outbreaks are waterborne but some are associated with contaminated food. Biophysical characterization of Norwalk virus indicates that its proteins resemble those of caliciviruses. Recently a serologically distinct 27 nm gastroenteritis virus, the Marin County virus, was successfully transmitted to adult volunteers. One volunteer shed large amounts of virus. Marin County virus shed by the volunteer was purified by ultracentrifugation techniques and used to make hyperimmune antisera which was used to develop a sensitive and specific RIA. Purified virus was characterized and shown to have 1 or 2 structural proteins (30 Kd). Monoclonal antibodies to purified Marin County virus are currently being isolated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00333-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A LONGITUDINAL STUDY OF VIRAL GASTROENTERITIS IN INFANTS AND YOUNG CHILDREN

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Albert Z. Kapikian, M.D. Head, Epidemiology Section LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	0.1	1.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rotaviruses have been studied extensively by predominantly cross-sectional approaches. Such studies have yielded essentially "numerator" data which indicated that rotaviruses are a major cause of diarrheal illness. There have been few longitudinal gastroenteritis studies yielding important epidemiologic information. Therefore we initiated an examination of anal swab and serum specimens obtained during a previous long-term longitudinal study (1955-1969) at Junior Village, a welfare institution for normal, homeless children. Anal swabs and blood specimens were obtained routinely. Surveillance was carried out by a trained medical staff. As reported previously, 139 rotavirus strains have been detected with the characteristic seasonal distribution. It should be possible to establish the serotypic diversity of these strains. The subgrouping pattern was consistent with other studies as most of the tested strains belong to subgroup 2. In addition, as noted previously, sequential sera from 384 children in residence sometime between May 19, 1963-May 31, 1966 have been tested for CF antibody to the "O" agent. 150 (40%) of the children experienced at least one rotavirus infection; 11 had a second infection and one a third infection. For the period from May 22, 1966-May 21, 1969 65 (36%) of 182 children (some overlap with previous period) experienced at least one rotavirus infection, with 6 having a second infection. We are currently attempting to propagate selected rotavirus positive specimens in tissue culture by direct isolation or genetic reassortment in order to serotype them.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00334-02 LID

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DEVELOPMENT OF MONOClonAL ANTIBODIES TO PROTEINS OF ROTAVIRUS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Harry Greenberg, M.D. Medical Officer LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
2.9 1.5 1.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In an attempt to clarify the nature of rotavirus antigens and as a means of investigating rotavirus protein structure and function we have isolated a series of monoclonal antibodies directed at several rotavirus proteins. Monoclonal antibodies were derived from mice immunized with the Wa strain of human rotavirus (serotype 1), or the rhesus 2 strain of simian rotavirus (RRV) or a human rotavirus (DS-1, serotype 2) reassortant (40-2). We studied 70 monoclonal antibodies directed at 5 viral proteins. Monoclonal antibody production utilized standard techniques and assay systems including RIA, neutralization, immunoprecipitation and hemagglutination-inhibition (HI). The monoclonal antibodies were characterized by the proteins with which they react: (1) monoclonals to the 42,000 dalton inner protein, the 6th gene product; two of these have subgroup specificity. (2) monoclonals to the 34,000 dalton surface glycoprotein, the 8th or 9th gene product; some of these neutralize the virus to high titer and some have HI activity. (3) monoclonals to the 82,000 dalton surface protein, the 4th gene product, the viral hemagglutinin; these monoclonal antibodies inhibit hemagglutination and neutralize virus. (4) monoclonals to the 28,000 dalton 10th gene product, the nonstructural glycoprotein; (5) one monoclonal that reacts with the 35 KD NS glycoprotein and several monoclonals that cannot be classified.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00335-02 LID

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

ROTAVIRUS REASSORTANTS: GENETICS AND USE IN ROTAVIRUS VACCINES

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Harry Greenberg, M.D. Medical Officer LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	0.5	1.7

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rotaviruses have a double stranded segmented RNA genome and hence undergo genetic reassortment at high frequency. We have produced a series of temperature sensitive mutants of both bovine (UK) and rhesus rotavirus. We have used such ts mutants to isolate reassortant rotaviruses from the growth yield of cells coinfecting with a variety of human rotaviruses and ts bovine rotavirus. These reassortants were used to ascertain the gene coding assignments for human rotavirus neutralization specificity and growth restriction in tissue culture. Also, the human-bovine rotavirus reassortants were used to define the serotypic diversity of human rotaviruses. We isolated a series of reassortants derived from cells coinfecting with ts bovine and ts rhesus rotaviruses. Characterization of these reassortants showed that: (1) neutralization specificity and hemagglutination reassort independently and are coded for by different genes (8 or 9 and 4 respectively); (2) protease enhancement of virus replication co-segregates with the viral hemagglutinin and is coded for by the same gene, i.e., gene 4. Finally, using highly specific hyperimmune or monoclonal antibodies, we have isolated a series of reassortants derived from a cross of wild type bovine (UK) or RRV with human rotavirus (serotype 1, 2, 3 or 4). These reassortants that were isolated in primary tissue, possess only one or two human rotavirus genes. One of these genes codes for the glycoprotein that induces neutralizing antibody and thus these reassortants represent prime candidates for use in a live attenuated vaccine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00346-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

HYBRIDIZATION STUDIES OF ROTAVIRUS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Jorge Flores, M.D.

Expert Consultant

LID NIAID

COOPERATING UNITS (if any)

Hospital de los Ninos, J.M. De Los Rios, Caracas, Venezuela (Dr. Perez, L. White)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:

0.5

PROFESSIONAL:

0.2

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have exploited the ability of rotaviruses to transcribe " in vitro " their genomic RNAs into single stranded (ss) RNAs to prepare radioactive probes that can be used in hybridization assays for detection of rotaviruses and for the analysis of the genetic relatedness among isolates of human and animal origin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00338-02 LID

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

CLONING OF ROTAVIRUS GENES

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Jorge Flores, M.D. Expert Consultant

LID, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MD. 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.2	1.2	1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have been able to make DNA copies from genes of three different rotavirus strains (WA, NCDV and Rh2) by reverse transcribing their genomic RNAs or single stranded RNAs synthesized *in vitro* from rotavirus particles. Tailing of those DNAs and insertion into PBR 322 plasmid has allowed their cloning into *E. coli*. The genes from which most of these clones derive have been identified by means of a dot hybridization assay. Clones containing copies of each rotavirus gene (except for genes 10 and 11) have been identified. The sizes of the rotavirus cDNA inserts into the plasmids have been determined for more than half of all the clones obtained (more than 2500). Rotavirus cDNAs that may represent full size copies of genes 5-9 have been identified and their restriction patterns analyzed. Some of them are currently being sequenced in order to obtain knowledge on the amino acid sequences of the proteins they code for and for developing a strategy for insertion into expression vectors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00339-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

CULTIVATION AND SEROTYPIC CHARACTERIZATION OF HUMAN AND ANIMAL ROTAVIRUSES

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Richard G. Wyatt, M.D.

Medical Officer

LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, BETHESDA, MARYLAND

TOTAL MANYEARS:

3.1

PROFESSIONAL:

1.4

OTHER:

1.7

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is designed to cultivate directly in cell culture a variety of human and animal rotavirus strains from diverse geographical areas and populations in order to define antigenic differences and to identify and develop potential vaccine candidates. Over 80 strains of human rotaviruses have been cultivated in MA104 or AGMK cells. Four distinct serotypes of human rotavirus have been identified and compared with each other and with 12 animal rotavirus strains. Three distinct serotypes represented only by animal rotavirus strains were defined, but 6 animal rotaviruses were also found which are serotypically similar if not identical to human rotavirus belonging to serotype 3 or 4. Potential candidate vaccine strains of different serotypes have been grown either in primary or secondary African green monkey kidney or in diploid cells which are suitable for vaccine production, and these now can be evaluated further for safety and efficacy.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00340-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

EXPERIMENTAL STUDIES IN ANIMALS WITH VARIOUS ROTAVIRUSES

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Richard G. Wyatt, M.D. Medical Officer LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:

0.8

PROFESSIONAL:

0.5

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Experimental animals were studied under gnotobiotic conditions or in isolation to evaluate virulence of selected human and animal rotavirus strains as well as to study homologous and heterologous immunity. Studies were performed primarily in gnotobiotic piglets and rhesus monkeys. Three potential vaccines or vaccine precursors (human rotavirus strain D, bovine rotavirus UK and rhesus rotavirus MMU18006) were evaluated in gnotobiotic piglets.

Infection in the absence of dehydrating diarrheal illness occurred following challenge with the human D strain and rhesus rotavirus. The UK bovine rotavirus did not cause disease; its capacity to infect piglets is not known at this time but this question will be answered when serologic studies are completed. These three strains will be studied further as potential vaccines. A member of the 4th human rotavirus serotype was also administered to gnotobiotic piglets; infection without dehydrating diarrheal illness was also observed. Prior exposure to rhesus rotavirus (serotype 3) appeared to decrease viral shedding following later challenge with human rotavirus strain M (serotype 3).

Rhesus rotavirus MMU18006 was also administered to juvenile rhesus monkeys and did not produce disease, although serologic evidence of infection was demonstrated. Additional safety testing of this strain to rule out the presence of an agent which produces acquired immune deficiency in rhesus monkeys is under way.

In a previous study of heterologous immunity, we demonstrated that in utero inoculation with bovine rotavirus protected calves against challenge with human rotavirus of a distinct serotype. Reevaluation of the calf sera and fecal samples from this study indicated that a single in utero exposure to bovine rotavirus induced broadly reactive neutralizing antibody and significantly diminished the viral shedding pattern on subsequent challenge with human rotavirus (strain D); this may explain the observed heterologous immunity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00341-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

STUDIES OF ROTAVIRUSES IN VOLUNTEERS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Richard G Wyatt, M.D.

Medical Officer

LID NIAID

COOPERATING UNITS (if any)

Center for Vaccine Development, U. of Md. (Dr. Michael Levine); NINCDS (Dr. William London); Smith-Kline-RIT (Belgium-Dr. Huygelen)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:

0.5

PROFESSIONAL:

0.3

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During a previous study, we observed that wild type human rotavirus, strain D, induced diarrheal illness in adult volunteers. Also in this study we identified serologic correlates of resistance. In a subsequent study, human rotavirus, Wa strain (type 1), adapted to growth in African green monkey kidney cell cultures was shown to infect susceptible volunteers. The failure of adult volunteers with a low level of pre-challenge serum antibody to develop diarrheal disease suggested that the Wa rotavirus mutant was attenuated compared to the wild type D rotavirus. This preparation of Wa strain cannot be used in further studies because a simian foamyvirus was subsequently identified in the seed used to prepare it. Also 3 volunteers developed a low-level rise in serum transaminase 10 days after administration of virus; the etiology of these rises has not yet been explained. Because of these problems, an additional suspension of the Wa human rotavirus is being prepared in pre-tested, adventitious virus-free African green monkey kidney cell cultures. It is planned that this virus suspension will be evaluated in susceptible adult volunteers. Efforts are also under way to adapt human rotaviruses to growth in human or rhesus monkey diploid cells; if successful, vaccines could be prepared in these virus-free cells.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00342-02 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

STUDIES OF GASTROENTERITIS VIRUSES BY ELECTRON MICROSCOPY

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Albert Z. Kapikian, M.D. Head, Epidemiology Section LID NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MARYLAND 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.83	0.66	0.17

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The electron microscope (EM) has been a mainstay for study of fastidious gastroenteritis viruses. Although 2nd and 3rd generation tests have been developed for the detection of "Norwalk" viruses and rotaviruses, EM remains an essential tool: (1) as the "supreme court" when newer tests yield variable results, (2) in the quest for new agents of viral gastroenteritis, (3) for visualizing the site of attachment of antibody on the virion in antigen-antibody reactions, (4) for serologic studies, (5) for direct visualization of virus particles, (6) for studying appropriate specimens derived from individuals with diseases of unknown etiology such as acquired immune deficiency syndrome (AIDS) and non-A, non-B hepatitis. The site of attachment of monoclonal antibody to the gene 8 product of rhesus rotavirus (serotype 3) was visualized to be on the outer capsid of the double shelled rotavirus particle. In addition, with such ascitic monoclonal antibody, several rotaviruses belonging to the third human rotavirus serotype could be easily serotyped by IEM. The site of attachment of monoclonal antibody to the gene 6 product of rhesus rotavirus was visualized to be on particles lacking the outer capsid (rough) but not on particles with an intact outer capsid (smooth). 27 nm particles were visualized in stools obtained from a large outbreak of gastroenteritis which was traced to contaminated cake frosting. Finally, about 50% of the episodes of pediatric diarrhea are still without any known etiology. A major effort should be made to examine by IEM or indirect IEM such "negative" specimens in an attempt to detect new etiologic agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00373-01 LID

PERIOD COVERED

October 1, 1982 through September 30, 1983

(Title of project (80 characters or less. Title must fit on one line between the borders.)

STUDIES FOR DETECTION OF ETIOLOGIC AGENT(S) OF AIDS BY IMMUNE ELECTRON MICROSCOPY

*(Principal investigator (List other professional personnel on subsequent pages.)**(Name, title, laboratory, and institute affiliation)*

Albert Z. Kapikian, M.D. Head, Epidemiology Section LID, NIAID

(Cooperating units (if any))

LAB/BRANCH

LABORATORY OF INFECTIOUS DISEASES

SECTION

EPIDEMIOLOGY SECTION

INSTITUTE AND LOCATION

NIAID, NIH, BETHESDA, MD 20205

TOTAL MANYEARS:

<.1

PROFESSIONAL:

< .1

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Acquired immune deficiency syndrome (AIDS) is a serious disease of major public health importance. Thus far, an etiologic agent has not been found. Immune electron microscopy, which is the direct observation of antigen-antibody interaction, has been employed as a means for detecting the fastidious etiologic agents of several diseases such as the 27 nm Norwalk agent of acute nonbacterial gastroenteritis, and the 27nm hepatitis A virus of hepatitis. Selected specimens from AIDS patients will be examined by immune electron microscopy or conventional electron microscopy in an attempt to detect the etiologic agent. Presently, collaborative studies using electron microscopy are being carried out on simian AIDS in collaboration with the NINCDS.

LABORATORY OF MICROBIAL IMMUNITY

1983 Annual Report
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PHS-NIH
SUMMARY STATEMENT

ANNUAL REPORT OF THE LABORATORY OF MICROBIAL IMMUNITY
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
October 1, 1982 to September 30, 1983

Richard Asofsky, M.D.
Chief, Laboratory of Microbial Immunity

Research in the Laboratory is concerned mainly with the differentiation of lymphoid cells and their neoplastic derivatives, and with the control of cellular and humoral immune responses. Neoplastic B-cell lymphomas are used to examine various stages of B cell differentiation. Somatic cell hybrids between normal B cells from different strains of mice and an HGPRT clone of one of the lymphomas are induced to differentiate to immunoglobulin secretion. Genetic studies of genes controlling lymphocyte differentiation, and neoplastic transformation, and of genes controlling certain antibody responses are in progress. Antigens which induce unresponsiveness very easily (polysaccharides), and regular cycling of antibody levels (lipopolysaccharides), and some which produce tissue damage and/or extensive "polyclonal" activation of lymphocytes (malaria) are used. Autoimmune diseases, such as the SLE - like syndrome of (NZB X NZW) hybrid mice and experimental encephalomyelitis are under active study.

SUPPRESSOR T CELL ACTIVITY IS EXPRESSED IN A CYCLIC MANNER

Prior treatment (priming) with a single injection of a subimmunogenic dose of Type III pneumococcal polysaccharide (SSS-III) results in an antigen - specific T-cell-dependent form of unresponsiveness (low-dose paralysis) mediated by suppressor T cells. Although such unresponsiveness persists for several months after priming, it is expressed in a cyclic manner with a periodicity of about 3 days. This cyclic pattern is accompanied by concurrent periods of Velban sensitivity that also are cyclic. This suggests that the maintenance of low-dose paralysis in part requires some degree of cell proliferation which proceeds in an ordered manner in response to a "signal" generated after priming with antigen. (P. J. Baker, C. E. Taylor, P. W. Stashak, G. Caldes, and M. Fauntleroy, LMI, NIAID; B. Prescott, Biomedical Research Institute, Rockville, MD.)

CELL-ASSOCIATED ANTIBODY PROVIDES A SIGNAL FOR THE ACTIVATION
OF SUPPRESSOR T CELLS

The transfer of B lymphocytes from mice immunized with Type III pneumococcal polysaccharide (SSS-III) results in antigen-specific suppression of the antibody response of recipients immunized with SSS-III. Such suppression shares many features associated with low-dose paralysis, a phenomenon mediated by suppressor T cells; it reaches maximal levels 3 days after the transfer of viable or irradiated immune B cells and can be eliminated by the depletion of SSS-III - binding cells from spleen cell suspensions prior to transfer. In a two-step cell transfer experiment, purified T lymphocytes, isolated from recipients previously given immune B cells, caused suppression upon transfer to other mice immunized with SSS-III. Also, B-cell-induced suppression could be abrogated in a competitive manner by the infusion of amplifier T lymphocytes.

These findings suggest that B cell surface components, presumably the idiotypic determinants of cell-associated antibody specific for SSS-III, are instrumental in activating suppressor T cells involved in regulating the magnitude of the antibody response to SSS-III. (P. J. Baker, C. E. Taylor, P. W. Stashak, G. Caldes and M. Fauntleroy, LMI, NIAID; B. Prescott, Biomedical Research Institute, Rockville, MD.)

SUBPOPULATIONS OF THYMUS CELLS IDENTIFIED BY MULTIPARAMETER CYTOMETRIC ANALYSIS

Thymocytes were stained with anti Lyt 1 and anti Lyt 2, with anti Lyt 2 and peanut lectin (PNA), and with other selected monoclonal antibodies. Cells were stained with one reagent labeled with fluorescein, a second labeled with a red fluorochrome, and were analyzed for green fluorescence, red fluorescence, and forward light scatter of each all. At least 4 subpopulations were revealed, one of which (phenotype: Ly 1 dull, Ly 2⁺, PNA intermediate) has not been described before. This population contains large cells as judged by light scatter. It is actively dividing and/or immature; it is perhaps an early precursor of thymocytes. These cells bear Ly 9 antigen, proving that they are lymphoid. Thymi obtained early after transplantation and thymi from animals recovering from treatment with steroids show enrichment of this cell during repopulation. Multiparameter cytometric analysis permits the identification and characterization of groups of cells not seen by other means. (Ms Fowlkes; Dr. Chused; Ms Edison (LMI); Dr. B. Mathieson (NCI); Mr. Leiserson (Basel Institute).

NULL CELLS IN NZB MICE

Investigation of lymphocyte populations of NZB mice has revealed that the increased percentage of "null" cells found in their spleens is composed of several different cell types, that appear to be regulated in concert by a single gene. In the backcross of NZB mice with (NZB x NZW) F₁, the null cell percentage in young mice was unrelated to the subsequent development of autoimmune disease, suggesting that the null cells are unrelated to the disordered immunoregulation of the NZB strain. During the development of disease in NZB mice, it was found that splenomegaly develops suddenly and is always accompanied by an increase in the size, but not proliferation rate, of the Ly-2⁺ T cells. (Dr. Chused, Mr. Leiserson, Ms Edison, LMI; Dr. Manohar).

DIFFERENTIATION STUDIED IN B CELL HYBRIDS

B-lymphocyte hybridomas, prepared by fusion of C57Bl/6 spleen cells with the HGPRT lymphoma M12.4.1 in vitro, were stimulated with affinity - purified anti-IgM, the phorbol ester, PMA, bacterial lipopolysaccharide, LPS, or with cytochalasin B (CB). Six different hybridoma lines differentiated to give 10-15% IgM-secreting cells after 3 days in culture with anti-IgM. Five of the six differentiated after exposure to LPS, two after exposure to PMA, and one after exposure to CB. The effect of anti-IgM, but not of the other stimulants was inhibited by the IgM myeloma protein, MOPC 104E; the effect of the PMA, but not the other stimulants was inhibited by retinoic acid. Cell lines which were differentiating, regardless of inducing agent, showed a 50% reduction in expression on cell membrane Ia antigens, and a slowing of cell division of about 50%. (Drs. Hamano and Asofsky, Mr. Leiserson, LMI; Dr. K. J. Kim (Revlon))

ADMINISTRATIVE

Dr. Teruaki Hamano returned to Japan in March, 1983 after 3 years of visiting fellowship. Dr. Christopher Bever, research associate joined NINCDS in July, 1982; he was replaced by Dr. Carol Sulis, medical staff fellow. Dr. John F. Finerty left the Laboratory for administrative duties. Dr. Jean Langhorne, his visiting fellow, is now sponsored by Dr. Asofsky. Dr. Chu Hsiao-Kun from the PRC joined LMI in February, 1983 as a guest worker. He is now a visiting fellow. Mr. Leiserson, the cytometer technician left in 1982 and was replaced by Ms Linette Edison.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00131-16 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Hypersensitivity in Inbred Histocompatible Guinea Pigs

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Sanford H. Stone, Chief, Experimental Autoimmunity Section, LMI, NIAID

COOPERATING UNITS (if any)

Clinical Branch and Laboratory of Vision Research, NEI; Division of
Neuropathology, Albert Einstein College of Medicine

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Autoimmunity Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In some demyelinating diseases of man, typified by multiple sclerosis (MS) the condition is frequently not determined until autopsy. This silent MS, which does not reflect the significant demyelination in the CNS, is worthy of investigation of the factors responsible for clinical protection in the face of putative severe immunological attack. The strain 2 guinea pig provides an age-, sex- and strain - dependent model for this situation in that female but not male juvenile animals sensitized with spinal cord antigens develop clinical experimental allergic encephalomyelitis (EAE), an established immunological animal model of MS, but both male and female guinea piglets show striking histological change and demyelination. Evidently, male juveniles are competent immunologically, but factors intervene to prevent the clinical course. Neither male nor female strain 2 juveniles show the cortical cataract of acute EAE found in this laboratory in strain 13's, but 13/2 hybrids which in contrast to strain 2 are susceptible to acute EAE, do manifest cataracts. An autosomal dominant nuclear cataract of strain 13 is being investigated for its particular crystallin production, genetics, phylogeny, histology, and biochemical and serological characteristics.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00134-21 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Control of Immunoglobulin Synthesis in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Richard Asofsky, Chief, LMI, NIAID

COOPERATING UNITS (if any)

*Laboratory of Immunology, NIAID

**Laboratory of Parasitic Diseases, NIAID

***Revlon Research Group, Springfield, NIAID

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

4.2

PROFESSIONAL:

2.8

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

B-lymphocyte hybridomas, prepared by fusion of C57Bl/6 spleen cells with the HGprt lymphoma M12.4.1 *in vitro*, were stimulated with affinity - purified anti-IgM, the phorbol ester, PMA, bacterial lipopolysaccharide, LPS, or with cytochalasin B (CB). Six different hybridoma lines differentiated to give 10-15% IgM-secreting cells after 3 days in culture with anti-IgM. Five of the six differentiated after exposure to LPS, two after exposure to PMA, and one after exposure to CB. The effect of anti-IgM, but not of the other stimulants was inhibited by the IgM myeloma protein, MOPC 104E; the effect of the PMA, but not the other stimulants was inhibited by retinoic acid. Cell lines which were differentiating, regardless of inducing agent, showed a 50% reduction in expression on cell membrane Ia antigens, and a slowing of cell division of about 50%.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00136-11 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization and Differentiation of Thymus Subpopulations

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

B. J. Fowlkes, Microbiologist, LMI, NIAID

COOPERATING UNITS (if any)

*Frederick Cancer Research Center, NCI

**Basel Institute of Immunology, Basel Switzerland

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.2

PROFESSIONAL:

0.3

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Expression of Lyt differentiation antigens has been examined on murine thymocytes by flow cytometry, using fluorochrome - labeled monoclonal antibodies to the antigens. Two-color flow cytometric analysis revealed at least 4 populations of cells, one of which has not been described before. The size, morphology, cell cycle kinetics and surface phenotype suggest that this cell is immature and/or dividing; it is perhaps an early precursor of more mature thymocytes. Thymus obtained early after transplantation, and thymi from animals recovering from treatment with steroids show enrichment of this cell during repopulation. These cells bear Ly 9 antigen, proving that they are lymphoid. Analysis with several other monoclonal antibodies shows a phenotype not shared by any peripheral cell examined. Although this population constitutes only 3-4% of the thymocyte population, it is readily isolated free from contaminants by multiparameter (2 color, red and green, and size) cell sorting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00137-16 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biology of Graft-Versus-Host Reactions

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Richard Aszofsky, Chief, LMI, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Inactive during current year.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00141-09 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immune Responses to Malaria and Related Intracellular Protozoa

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Jean Langhorne, Visiting Fellow, LMI, NIAID

COOPERATING UNITS (If any)

D. W. Taylor, Department of Biology
Georgetown University, Washington, D.C.

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The study of humoral response of mice to malaria antigens was continued. The work was extended to include analyses of Plasmodium chabaudi infections in addition to P. yoelii. During the year the antigenic cross-reactivity between P. chabaudi and P. yoelii and the humoral responses to the cross-reactive antigens were compared. Cross reactivity at the antibody level was found to be extensive. Further, the antibody response to the cross reactive antigens in the two infections differed with regard to isotype distribution. It was also found that cross reactive antigens can be present on molecules of different size in the two species.

In order to examine T cell regulation and control of malaria specific antibody responses during infection, experiments were initiated to establish conditions for the in vitro generation of such antibody responses T cell lines are being developed by the use of hybridoma techniques and continuous in vitro antigen stimulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00142-09 LMI

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Development of Thymus-derived (T) Suppressor and Amplifier Cell Function

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)
P. J. Baker, Head, Microbiology and Immunology Section, LMI, NIAID

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Microbial Immunity

SECTION
Microbiology and Immunology Section

INSTITUTE AND LOCATION
NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Inactive during current year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00143-14 LMI
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic Control of the Antibody Response to Type III Pneumococcal Polysaccharides		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and Institute affiliation) P. J. Baker, Head, Microbiology and Immunology, LMI, NIAID		
COOPERATING UNITS (If any) *Service de Chimie-Physique, Campus Plaine ULB, Brussels, Belgium **Biomedical Research Institute, Rockville, MD ***Abbott Laboratories, North Chicago, IL		
LAB/BRANCH Laboratory of Microbial Immunity		
SECTION Microbiology and Immunology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>Several different strains of mice that give low - or no - antibody responses to bacterial polysaccharide antigens such as Type III pneumococcal polysaccharide (SSS-III), the lipopolysaccharide of <u>Escherichia coli</u> 0113 (LPS 0113) and $\alpha 1 \rightarrow 3$ dextran of <u>Leuconostoc mesenteroides</u>, were crossed to mice that usually give high responses to these antigens in order to characterize the types of genetic control mechanisms involved in regulating antibody responses and their linkage to known genetic loci. The genetic defects of C3H/HeJ, CBA/N and RIII S/J mice were found to be unrelated (unlinked). Crosses between these strains and other high responding strains of mice provided evidence for genetic complementation and the involvement of multiple genes, none of which appear to be linked to the H-2 or IgC_H allotype loci.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00144-19 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of the Antibody Response to Microbial Polysaccharide Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

P. J. Baker, Head, Microbiology & Immunology, Section, LMI, NIAID

COOPERATING UNITS (if any)

*Biomedical Research Institute, Rockville, MD 20205

**Service de chimie-Physique, Campus Plaine ULB, Brussels, Belgium

***Abbott Laboratories, North Chicago, IL 60064

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Microbiology & Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.6

PROFESSIONAL:

0.3

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

AKR/N and BALB/c Cum mice differ greatly with respect to their capacity to develop immunological memory to lipopolysaccharide (LPS) derived from Escherichia coli 0113 (LPS 0113). Substantial memory can be induced in BALB/c Cum mice primed with extremely low doses (10^{-5} μ g) LPS 0113; however, only a modest degree - at best - of memory is induced in AKR/N mice, regardless of the dose of LPS 0113 used for priming. Since the generation of memory to LPS 0113 in BALB/c Cum mice proceeds in a cyclic manner, memory may play an important role in the expression of a cyclic antibody response to this antigen.

Other studies on the antibody response to Type III pneumococcal polysaccharide have shown that the expression of suppressor T cell activity is cyclic with a periodicity of about 3 days; the kinetic pattern obtained is associated with concurrent stages of Velban sensitivity that likewise are cyclic. This implies that suppressor T cells proliferate in an ordered manner in response to a "specific signal" generated after exposure to antigen.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00145-16 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mode of Action of Thymus-derived (T) Suppressor & Amplifier Cells

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

P. J. Baker, Head, Microbiology and Immunology Section, LMI, NIAID

COOPERATING UNITS (if any)

*Biomedical Research Institute, Rockville, MD 20205

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Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.9

PROFESSIONAL:

1.2

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The transfer of purified B lymphocytes from mice immunized (or primed) with Type III pneumococcal polysaccharide (SSS-III) results in the induction of antigen-specific suppression of the antibody response of recipients immunized with SSS-III. Such suppression shares many features associated with low-dose paralysis, a phenomenon mediated by suppressor T cells; it reaches maximal levels 3 days after the transfer of viable or irradiated immune B cells. In a two-step cell transfer experiment, purified T lymphocytes, isolated from recipients previously given immune B cells, caused suppression upon transfer to other mice immunized with SSS-III. Also, B-cell-induced suppression could be abrogated in a competitive manner by the infusion of amplifier T lymphocytes. These findings suggest that B cell surface components, presumably the idiotypic determinants of cell-associated antibody specific for SSS-III, are instrumental in activating suppressor T cells involved in regulating the magnitude of the antibody response to SSS-III.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00146-10 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunological Studies of Components Isolated from Bacteria, Parasites and Plants

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

G. Caldes, Chemist, LMI, NIAID

COOPERATING UNITS (if any)

*Biomedical Research Institute, Rockville, MD 20205

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Laboratory of Microbial Immunity

SECTION

Microbiology and Immunology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.1	0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Polysaccharides were isolated from two fungi, Auricularia Wood's-ear and Flamulina velutipes, and one medicinal plant, Amaracus dictamnus, which cross-reacted immunologically with Type III pneumococcal polysaccharide. Mild acid hydrolysis and chromatographic separation with D.E.A.E. Sephadex yielded polysaccharides of increased activity in Ouchterlony tests with Type III pneumococcal antiserum.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00153-06 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In vitro Response of Human Peripheral Lymphocytes to Infectious Organisms

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Richard Asofsky, Chief, LMI, NIAID

COOPERATING UNITS (If any)

None

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Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Inactive during current year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00186-10 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Autoimmunity in Inbred Strains of Mice

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Thomas M. Chused, Senior Investigator, LMI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.0

PROFESSIONAL:

1.4

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The immunologic mechanism and genetic control of autoimmune disease is being investigated in autoimmune mice utilizing flow microfluorometry and functional analysis of lymphocyte subpopulations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00187-09 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies in Sjogren's Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Thomas, M. Chused, M.D., LMI, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Inactive during current year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00203-04 LMI

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Applications of FFlow Microfluorometry

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Thomas M. Chused, Senior Investigator, LMI, NIAID

COOPERATING UNITS (if any)

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Laboratory of Microbial Immunity

SECTION

Experimental Pathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.6	1.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Flow microfluorometry, supported by multiparameter data analysis is being applied to problems in multiple areas: 1) regulation of membrane potential by lymphocytes and neutrophils, 2) lymphocyte ontogeny and activation, 3) abnormalities in lymphocyte subpopulations associated with murine autoimmune disease, and 4) lymphocyte and monocyte analysis in human immunologic diseases, including systemic lupus erythematosus and acquired immunodeficiency syndrome (AIDS).

LABORATORY OF MOLECULAR MICROBIOLOGY
1983 Annual Report
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PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR MICROBIOLOGY, NIAID
October 1, 1982 - September 30, 1983

Dr. Malcolm A. Martin
Chief, Laboratory of Molecular Microbiology

The Laboratory of Molecular Microbiology (LMM) applies molecular biological techniques to study the structure and regulation of prokaryotic and eukaryotic genes. Our research goals are to answer fundamental questions in microbiology by examining host cells and associated microorganisms at the molecular level. While a great deal of effort is directed to animal virus systems, bacterial and mycoplasma organisms are also investigated. Relying heavily on such biochemical techniques as nucleic acid hybridization, restriction enzyme mapping, DNA cloning, and nucleotide sequencing procedures, LMM staff members have productively investigated a variety of genes involved in the interaction of a particular microorganism and its host cell. In many cases, newer technologies have been combined with more conventional assay systems, particularly following the construction of novel and potentially active DNA recombinants.

During the past year, considerable progress has been made in several different areas. Many members of the Biochemical Virology Section have had a long-standing interest in retroviruses. The focus of these investigations has been the detailed analysis of endogenous proviral DNA found in mice and man. Although retrovirus particles contain an RNA genome, a DNA copy of the viral RNA is synthesized following virus infection, becomes integrated into the host-cell chromosome, and is the template from which messenger and genomic RNA is copied. The genome of normal vertebrate cells contains multiple copies of retrovirus DNA which are vertically transmitted. In the mouse and avian systems, some of these copies of retroviral DNA (so-called "proviruses") are expressed as competent, infectious virus particles. Most of these "endogenous" copies of retroviral DNA, however, do not give rise to infectious virions since they contain multiple deletions and rearrangements. The status and function of the more than 50 copies of proviral DNA in normal or diseased cells are presently not understood. It is our goal, using molecular biological techniques, to begin unraveling the function of this multi-copy family of genes. Our studies in this area were initiated about four years ago as a result of collaborative arrangements made with Dr. Wallace Rowe and his colleagues in the Laboratory of Viral Diseases (LVD, NIAID). During this period, considerable progress was made in the biochemical characterization of the different classes of endogenous murine leukemia virus (MuLV) sequences present in the mouse genome. The success of these experiments was in a large part due to Dr. Rowe's interest and leadership, particularly during the early phases of these studies. His important contributions to our research program in this area will be sorely missed.

One model of retrovirus-induced leukemogenesis involves the generation of dual-tropic MuLVs following recombination between spontaneously induced ecotropic MuLVs and endogenous proviral DNA segments present in mouse chromosomal DNA. Subsequent to the recombination event, the dual-tropic MuLVs may gain entry into a susceptible cell (such as a lymphocyte in the thymus) and, if integration of a proviral DNA copy occurs at an appropriate site (near a putative oncogene), disease may occur. A major determinant of cell tropism for retroviruses resides in

the envelope (env) glycoprotein. During the past year, the nucleotide sequence of several different MuLV env regions has been determined. The result of this analysis indicated that the env genes of some endogenous MuLVs were indistinguishable from analogous DNA segments of dual-tropic MuLVs and points to endogenous proviral DNA as a progenitor (via molecular recombination) of dual-tropic MuLVs. A 16 bp MCF-specific DNA oligonucleotide was synthesized in collaboration with Drs. Salzman and Rodi (LBV) and used to monitor the presence of dual-tropic envelopes in cloned and genomic DNA preparations. In another group of experiments, nucleotide sequence analyses revealed the presence of a unique 190 bp segment, inserted at identical locations, in four endogenous long-terminal repeats (LTRs) examined. The functional significance of this large insertion into an important regulatory region is currently being evaluated by constructing and assaying a series of recombinants containing the unique LTR element. Other recombinant MuLVs, containing portions of the env gene derived from different endogenous MuLV segments, are also being tested for alterations in host-range and leukemogenicity.

Similarly organized, endogenous type C retroviral DNA has also been cloned from a human gene library. These sequences can be grouped into two classes: approximately 25% have the properties of a full-length type C retroviral DNA and contain LTR, gag, pol, and env regions. Nucleotide sequencing has revealed the presence of several characteristic features of retroviral LTRs including inverted repeats, regulatory signals involved in transcription, and the presence of an adjacent polypurine tract (near the 3' LTR) and a putative t-RNA binding site (adjacent to the 5' LTR). Extensive nucleotide and amino acid sequence conservation of the gag and pol regions of human and murine proviral DNAs was observed. The regions of greatest polynucleotide sequence homology are situated in the p30 gag and pol genes. Weaker homology was detected at the deduced amino acid level between the p15 and p10 gag segments. The human env region has a large open reading frame with six potential glycosylation sites but shares no homology with comparable regions of any known retrovirus. Normal and malignant human tissues have been screened for the presence of retrovirus-related RNAs using radiolabeled DNA probes derived from the LTR, gag, pol, and envelope segments of the cloned human endogenous retroviral DNAs. Liver cells, B cell lines, and several human carcinoma lines contained no detectable RNA. Conversely, a tissue-specific pattern of expression has been observed employing RNAs prepared from human spleen, placenta, colon carcinoma, T cell leukemia, retinoblastoma, and rhabdomyosarcoma cells. Northern blot hybridization analyses indicated that two different human placentas contained substantial amounts of a 3.0 kb RNA species that anneals to LTR and env DNA probes. The reactive RNA comigrated in a similar gel with a 21S spliced MuLV LTR-env RNA isolated from virus-producing cells. Variable amounts of env and LTR-reactive poly A⁺ RNAs were present in a human colon carcinoma line. Additional RNA dot blot and Northern analyses are in progress in a survey to evaluate a wider range of human cell types including those from a variety of hematopoietic diseases.

Having sequenced more than 7200 bp of cloned retrovirus DNA, we determined the deduced amino acid sequence of putative human retroviral proteins. Hydrophilic regions from the pol and env genes have been identified, and oligopeptides corresponding to these regions are being synthesized in collaboration with Drs. John Coligan and Lee Maloy (LIG). Rabbit antisera directed against these peptides will be used to identify related proteins in normal and diseased human tissues.

Rapid progress continues to be made in two different papovavirus systems. Two months following exposure to a B cell lymphotropic papovavirus (LPV), originally isolated from the African green monkey, foci of morphologically transformed primary hamster embryo cells were detected. These cells contained intranuclear tumor (T) antigens that reacted with anti-LPV antiserum by the FA test. These cells also reacted weakly with T antiserum prepared against SV40, BK, and JC viruses, suggesting an evolutionary relationship between the LPV T antigen and the transforming proteins of other primate polyoma viruses. These studies represent the first successful demonstration that LPV is capable of transforming mammalian cells. Immunoprecipitation of 35 S-methionine-labeled LPV transformed cell extracts revealed an 84K protein that comigrated with an early viral gene product present in lytically infected cells. The tumorigenicity of the LPV transformed cells in newborn and weanling hamsters is currently being evaluated. Unlike SV40, the LPV transformed hamster cells contain unintegrated copies of the viral genome.

Experiments dealing with polyoma virus (PV) middle T antigen, the viral-encoded transforming gene product, indicated that its phosphorylating activity (of tyrosine residues) could be stimulated by epidermal growth factor (EGF). These in vitro experiments, utilizing purified plasma membranes from virus transformed cells, support a model involving the activation of middle T antigen by extra-cellular mitogenic growth factors. Recombinant plasmids were constructed for the expression of PV middle T antigen in E. coli in order to produce large quantities of the unmodified middle T polypeptide. A fused protein containing 23 amino acids of the O gene (of λ phage) product attached to the 21st amino acid of middle T antigen was synthesized in E. coli. Unfortunately, the immunoprecipitated fused proteins were not active in the phosphorylation assay nor was its tyrosine residue phosphorylated.

Work has also continued in the area of the tumor-inducing capacities of hamster cells transformed by human adenoviruses, simian virus 40, adeno-SV40 recombinant viruses, and polyomavirus, investigating in each case mechanisms of tumor cell establishment and rejection. Tumor induction by transformed somatic cell hybrids that expressed both adenovirus 2 and SV40 T antigens was reduced in adult syngeneic hamsters and absent in adult allogeneic animals compared to cells expressing only SV40 T antigen. Similarly, these same hybrids exhibited an increased cytolytic susceptibility measured in an in vitro assay compared to analogous experiments carried out with SV40 transformed hamster cells. Both results suggest that the tumorigenic phenotype and resistance to effector cell lysis are inversely related to the expression of some adenovirus 2 gene product. In another group of experiments carried out in collaboration with Dr. Heiner Westphal (NICHHD), data were obtained suggesting that the presence SV40 splicing sites in certain adeno-SV40 recombinant viruses interferred with their propagation in monkey cells. This could be due to incorrect processing of the adenovirus fiber mRNA which encodes an indispensable virus protein since its gene is located immediately downstream from the inserted SV40 DNA segment in the hybrid virus genome.

A major goal of the Bacterial Virulence Section is to assess the role of plasmids and plasmid-mediated genetic traits in the ecology, taxonomic status, and pathogenic potential of streptococci. Among the known plasmid-associated properties of streptococci, some appear to be limited to certain serological groups while others have been disseminated among virtually all species of the genus. Research efforts have focused on the characterization of representative "narrow

host-range" and "broad host-range" streptococcal plasmids. During the past year, a detailed restriction map of the 80.7 kb narrow host-range plasmid JH1 and the location of the kanamycin, streptomycin, erythromycin, and tetracycline resistance determinants was determined. The erythromycin resistance gene was shown to be located on a transposable element, Tn917. Several reports of the isolation of *S. fecalis* strains from clinical specimens with antibiotic resistance patterns identical or similar to that associated with strains JH1 have appeared in the literature. Employing nucleic acid hybridization and restriction mapping techniques, results have been obtained indicating that Tn917 has been disseminated among *S. fecalis* strains in swine, chickens, and humans over a wide geographic area.

During the past year, a detailed restriction endonuclease map of the broad host-range transmissible plasmid pAMB1 was deduced and a 5 kb Eco RI restriction fragment containing replication function(s) was identified. The latter Eco RI fragment was ligated to the *E. coli* plasmid vector pACK1 and the resulting recombinant replicated in both *E. coli* and streptococcal host cells. The streptococcal replicon has been further localized to a 2.95 kb Hind III + Eco RI fragment. This streptococcal replicon is being manipulated as a potential cloning vector for gram-positive bacteria.

The obligate anaerobic bacteria of the *Bacteroides fragilis* group are the predominant members (approximately 25% of the intestinal microflora of man). These organisms play an important role in the normal functions of the gastrointestinal tract. In addition, they can also be significant opportunistic pathogens frequently isolated from patients with intra-abdominal sepsis, bacteremia, and brain abscesses. The *Bacteroides*, as a group, are somewhat refractory to chemotherapy; they are inherently resistant to B-lactam and aminoglycoside antibiotics and, during the past decade, have developed resistance to tetracyclines. During the past year, studies were initiated to evaluate the transfer of clindamycin resistance among *Bacteroides*. Two large (approximately 47 and 85 kb) plasmid DNAs were identified and are currently being characterized. In a second group of experiments the transmission of tetracycline resistance between two clinical *Bacteroides* isolates is also being studied. Preliminary results indicate that drug-resistant donors and recipients contain a common 2.7 kb plasmid.

The new mycoplasma recovered from the urogenital tract of patients with nongonococcal urethritis (*M. genitalium*) was further characterized. This organism shares antigenic relationships with *M. pneumoniae*, presumably because of evolutionarily related surface antigens. Following the inoculation of chimpanzees with the G-37 strain of *M. genitalium*, 10^5 - 10^7 organisms were shed for 8 to 12 weeks. In addition, evidence for systemic infection following this challenge was suggested by the recovery of the G-37 organism from the blood of two of the chimpanzees at 3 to 7 weeks postchallenge. A new mycoplasma (*M. muris*) was isolated from the vaginal tracts of pregnant RIII mice utilizing the SP-4 culture media developed in our laboratory for the cultivation of spiroplasmas (helical mycoplasmas). This medium can improve the isolation of *M. hominis* from human genital tract specimens by 30% in females to 45% in males compared to conventional culturing techniques.

Specific Research Accomplishments

Structural and functional analysis of endogenous MuLV DNA sequences from AKR/J and BALB/c mice. Twelve endogenous MuLV DNA clones were isolated (eight from a BALB/c mouse embryo genomic library and four from shot-gun cloning of size-selected AKR/J mouse DNA), all of which contained LTR elements and associated gag, pol, and env genes. The nucleotide sequence of five endogenous MuLV LTRs was determined: in all cases the transcriptionally important regulatory signals were conserved. A unique feature in four of the endogenous LTRs was the presence of a highly conserved 190 bp insert located, in each case, 48 bp upstream from the C-C-A-A-T sequence. The role of this insert in modulating the functional activity of the endogenous LTRs is currently being studied. (Khan and Martin)

Five of the MuLV DNA clones contained env sequences. Four of the endogenous env segments contained restriction sites and hybridization properties unique to exogenous MCF MuLVs. Nucleotide sequence analysis of one endogenous envelope region and the env gene of the dual-tropic MCF 247 MuLV indicated that the two were virtually identical in the 5' env region. A comparison of the nucleotide sequence in the 3' pol and 5' env segments in MCF247 and xenotropic MuLV DNAs allowed the identification of sequences unique to recombinant dual-tropic MuLVs. They include (i) a 12 bp insertion in 3' region of pol and (ii) several amino acid substitutions and deletion of four amino acids in the 5' region of env. These structural characteristics, which are unique to MCF, were present in the env region of the endogenous clone A-12. (Khan)

Characterization of LTRs associated with an endogenous retroviral segment cloned from African green monkey DNA. Several MuLV-related DNA clones were isolated from an African green monkey (AGM) genomic library. Restriction enzyme and hybridization analysis indicated that the AGM retroviral clone was closely related to the type C endogenous virus of the baboon in the gag, pol, and env regions, whereas the LTRs were different. Nucleotide sequencing of the AGM LTR demonstrated conservation of such regulatory signals as the CCAAT and TATA sequences. Other than this, little if any polynucleotide sequence homology was observed with the LTR of the baboon endogenous virus. The 3' AGM LTR segment exhibited enhancement activity as measured in chloramphenicol acetyl transferase gene assay. Studies are currently in progress to investigate the functional activity of the 5' LTR associated with AGM proviral DNA. (Khan)

The endogenous ecotropic provirus of BALB/c mice has been cloned. Results of Southern blotting experiments indicated that the single copy of ecotropic proviral DNA present in BALB/c mice was located on a 21 kb Eco RI cleavage product. Attempts to clone this size-fractionated segment using cosmid and Charon vectors were initially unsuccessful. Five positive recombinants were isolated, however, following the screening of 1.5×10^6 phage from a BALB/c genomic library. All hybridized to an ecotropic MuLV envelope-specific DNA probe constructed in our laboratory and contained the 3' LTR as well as up to 5 kb of adjacent pol and env sequences. Following transfer to a plasmid vector, a) functional studies involving its transfection into susceptible cells and b) nucleotide sequence comparison of its LTR segment with those of other MuLVs will be carried out. (Theodore and Martin)

Cloning of an infectious xenotropic MuLV. The first reported infectious xenotropic MuLV has been cloned from mink cells infected with virus induced from NZB mouse cells. Infectivity of the cloned MuLV was determined by immunofluorescence, by S^+L^- complementation, and by passage of virus in culture supernatant from infected cells. Additional studies on host-range specificity of MuLVs are now possible with the availability of this agent. Comparisons of sequences in the env gene of ecotropic, dual-tropic, and this new cloned xenotropic MuLV should indicate which sequences determine host-range. (Repaske)

Human DNA contains at least two families of endogenous retroviral sequences. Characterization of MuLV-related human endogenous retroviral clones by restriction mapping, Southern blotting, and partial nucleotide sequence has revealed the existence of two major families of retroviral structures. One family, comprising one-fourth of nearly forty human clones isolated to date, consists of full-length (8.8 kb) proviruses. Partial nucleotide sequencing indicated interrupted but substantial collinear identity between the deduced amino acid sequence and that of Moloney MuLV, extending from p15 gag to late pol (including the splice acceptor site for env mRNA). Conservation is greatest in pol; homology also exists in N-terminal p30 gag, a region which is highly conserved among mammalian retroviruses. Both LTRs from two clones have been sequenced; they are 494-503 bp in length, with inverted repeats and putative regulatory signals. The 3' LTR is preceded by a polypurine tract and the 5' LTR followed by a potential t-RNA binding site. There is 94% sequence homology between 5' and 3' LTRs of one clone but only 78% sequence homology exists between the two 5' LTRs.

The second family of endogenous retroviral segments comprises the remainder (approximately three-fourths) of the human clones, and are homologous with the full-length proviruses along a continuous 4.1 kb stretch extending from mid-gag (equivalent to p12 of Moloney MuLV) to late pol. Unlike the first family, however, these clones lack LTRs or putative env sequences. The 4.1 kb of retroviral sequence is flanked by a tandem array of imperfect 74 bp repeats, numbering eight at the 5' end and at least twelve at the 3' end in the two clones examined. (Repaske, Steele, Rabson, Martin)

Organization and expression of full-length human endogenous retroviral DNA. Full-length (8.8 kb) endogenous retrovirus segments have been identified in human genomic DNA. These DNA segments contain LTR sequences approximately 500 bp in length, gag and pol regions exhibiting extensive nucleotide and deduced amino acid sequence homology with corresponding regions of Moloney MuLV, and a putative env gene containing a long open reading frame lying between a potential env mRNA splice acceptor site and the 3' LTR. To assess whether expression of endogenous retroviral sequences occurs in human cells, DNA:RNA hybridization studies have been carried out employing specific subgenomic DNA segments. A 3.4 kb poly A⁺ RNA species was identified in human placenta that hybridized to LTR and env sequences. Human rhabdomyosarcoma cells contained a 5.4 kb RNA reactive with probes derived from LTR, gag, and pol regions. Multiple additional human tissues and cell lines were assayed for the presence of RNA related to the cloned endogenous retroviral sequences by the dot hybridization technique. Normal spleen, retinoblastoma, colon carcinoma, and leukemic T cells contained RNA that annealed to various retroviral DNA segments; however, no hybridization of liver or B lymphocyte RNAs was observed. These results indicate that endogenous retroviral sequences are differentially expressed in a variety of normal and neoplastic human tissues. (Rabson, Steele, and Martin)

The lymphotropic papovavirus isolated from the African green monkey has transforming activity. The transforming capacity of lymphotropic papovavirus (LPV) was demonstrated in hamster embryo cells. LPV transformed cells exhibited typical phenotypic cell properties (growth in soft agar and low serum) and also contained intranuclear T antigens which crossreacted weakly with anti-SV40, BK, and JC T antiserum. Immunoprecipitation of the T antigens of LPV showed that the large T antigen was an 84K protein (similar in size to that previously found in lytically infected cells) and a 17K small T antigen. Integrated as well as free viral DNA was found in transformed cells. (Takemoto and Kanda)

Enhancement of tyrosine phosphorylation of middle T antigen by epidermal growth factor (EGF). Previous results suggested that cellular kinases may be involved in the activity/activation of polyomavirus middle T (MT) antigen kinase. During a search for such cellular kinases, it was discovered that epidermal growth factor (EGF) enhances tyrosine phosphorylation of middle T antigen, presumably via the EGF receptor (a tyrosine kinase). These results suggest that the transformed phenotype may be regulated by extracellular mitogenic growth factors (such as EGF) by modulating the activity of the tyrosine kinase which phosphorylates middle T antigen. (Segawa and Ito)

Antiserum against a synthetic polypeptide representing the unique region of middle T antigen blocks the protein kinase activity. By further characterizing antibodies directed against the putative phosphorylation site of polyomavirus middle T antigen, it was found that they crossreacted with a 130K cellular protein and microfilament bundles of untransformed cells. Although the fluorescent labeling pattern (of microfilament bundles) is indistinguishable from that obtained by anti-actin antibodies, the antipeptide antibodies failed to immunoprecipitate actin. It is not presently known whether the 130K protein is associated with microfilaments. The 130K protein is neither vinculin nor the myosine light chain kinase. (Ito)

Suppression of the simian virus 40 tumorigenic phenotype in hybrid cells formed from simian virus 40 and adenovirus 2 transformed hamster embryo cells. Hamster cells transformed by Ad2 or SV40 have different tumorigenic phenotypes. Somatic cell hybrids formed from Ad2 and SV40 transformed hamster cells were used to determine whether possible interactions between the integrated viral genomes would influence the tumorigenic phenotype of hybrid transformed cells. These somatic cell hybrids were of two types, one expression both Ad2 and SV40 T antigens and the other expression only SV40 T antigens. Tumor induction by hybrid cells that expressed both Ad2 and SV40 T antigens was reduced in adult syngeneic hamsters and abrogated in adult allogeneic hamsters. These results indicate that the tumorigenic phenotype of transformed somatic cell hybrids that contain both the Ad2 and SV40 genome is governed by the genetic expression of Ad2. This expression may alter the ability of SV40 transformed hamster cells to resist the immunologically nonspecific defenses of the host. (Lewis)

Adenovirus 2 early gene expression promotes susceptibility to effector cell lysis of hybrids formed between adenovirus 2 and simian virus 40 transformed hamster cells. Weakly oncogenic Ad2 transformed LSH hamster cells are sensitive to lysis by spontaneously cytolytic lymphoid cells and activated macrophages, while highly oncogenic SV40 transformed LSH cells are relatively resistant to these nonspecific effector cells. Somatic cell hybrids formed

between Ad2 and SV40 transformed hamster cells, which expressed Ad2 T antigen, exhibited an increased cytolytic susceptibility compared to Ad2 T antigen negative cell hybrids or nonhybrid SV40 transformed cells. No correlation was found between the expression of SV40 T antigen in hybrid cells and cytolytic susceptibility. The results suggest the existence of a novel function for early Ad2 genome-encoded polypeptides (T antigens) expressed in transformed hamster cells--the induction of susceptibility to immunologically nonspecific effector cell-mediated destruction. (Lewis)

The Ad2-SV40 hybrid virus Ad2⁺ND₄ requires deletion variants to grow in monkey cells. By studying the kinetics of plaque induction in continuous lines (BSC-1 and CV-1) of the monkey cells, we have found that stocks of Ad2⁺ND₄ that were considered (from plaque purification studies and the kinetics of plaque induction in human and primary monkey cells) to be homogenous populations of Ad2⁺ND₄ virions are actually heterogeneous populations of Ad2⁺ND₄ virions and Ad2⁺ND₄ deletion variants that lack SV40 and frequently Ad2 DNA sequences at the left Ad-SV40 junction within the recombinant viral genome. Due to the defectiveness of the Ad2⁺ND₄ virus, the production of progeny in BSC-1 and CV-1 cells requires complementation between the Ad2⁺ND₄ genome and the genome of an Ad2⁺ND₄ deletion variant. We have concluded that the Ad2⁺ND₄ deletion variants are providing a component, possibly the fiber protein, that is essential for the production of Ad2⁺ND₄ progeny. These data imply that the Ad2⁺ND₄ virus is incapable of replicating in singly infected primary monkey cells without generating deletion variants. (Lewis)

Experimental infections with Mycoplasma genitalium. Further work on an experimental intraurethral challenge of chimpanzees with the new human genital mycoplasma has confirmed the occurrence of a persistent urethral infection and specific antibody response. New evidence for the invasiveness of the organism was found in the recovery of M. genitalium from blood cultures of two of four animals shedding large numbers (10^5 to 10^7) of organisms from the urethra. Additional trials also confirmed the ability of the organism to produce persistent colonization and inflammatory responses in the lower genital tract of female monkeys. (Tully)

Genetic characteristics of various mollicutes (wall-free prokaryotes). Comparative analysis of the genetic relatedness among established species of three groups of mollicutes (mycoplasmas, acholeplasmas, spiroplasmas) utilized DNA-DNA hybridization techniques and restriction enzyme digestions of mycoplasmal DNAs. The data offered support for current species distinctions based primarily upon phenotypic markers. Organisms with strict host and tissue specificity (such as some Mycoplasma species) showed marked genotypic homogeneity, while species recovered from a variety of hosts or habitats (such as Acholeplasma species) are more heterogeneous in genetic characteristics. (Tully)

Epidemiology of β-hemolytic Streptococcus faecalis infections. Five restriction endonucleases, Eco RI, Xba I, Bam HI, Sal I and Xho I, yielding 10, 9, 3, 2, and 2 fragments, respectively, were used to determine the size (80.7 kb) and generate a physical map of the narrow host-range streptococcal R plasmid pJH1. Hybrid plasmids containing segments of pJH1 DNA were used for the localization of kanamycin, streptomycin, erythromycin and tetracycline resistance genes. The erythromycin resistance determinant of pJH1 was shown to be contained in the transposon Tn917, an erythromycin resistance transposon originally

reported to be associated with another *S. faecalis* R plasmid. This latter plasmid, pAd2, which also carries the same kanamycin and streptomycin resistance determinants as pJH1, was shown to be homologous to two contiguous segments, separated by 4 kb, of pJH1. pAD2 is most likely a deletion derivative of this plasmid. (LeBlanc and Banai)

A comparative analysis of multiple antibiotic resistance plasmids from *S. faecalis* strains of human and animal origin. Eleven conjugative plasmids, mediating the same antibiotic resistance pattern as pJH1 and present in *S. faecalis* isolated from human clinical specimens and the feces of healthy farm animals, were shown to share between 50% and 90% of their DNA sequences with pJH1. The erythromycin resistance determinant of 5 of the 11 plasmids was shown to be present in the transposable element Tn917. The 5 plasmids were harbored by *S. faecalis* strains isolated from the feces of a healthy pig in Illinois, a broiler in North Carolina, a broiler in Arkansas, and from clinical specimens obtained from two human patients in Washington, DC. These data suggested that Tn917 has been disseminated among human and animal *S. faecalis* strains over a wide geographical area. (LeBlanc and Rollins)

Molecular and genetic approaches to the study of anaerobic bacteria and their plasmids. A research program dealing with antibiotic resistance transfer among the obligate anaerobic bacteria of the Bacteroides fragilis group has been established. An 85 kb clindamycin resistance plasmid, pIB136, has been discovered in a clinical isolate of *B. ovatus* and progress is being made toward a detailed physical analysis of this plasmid. Several tetracycline resistant Bacteroides species have been obtained and found to possess the ability to transfer this resistance phenotype by conjugation. Efforts are underway to determine the nature of the genetic element(s) responsible for conjugative transfer in these organisms. A survey of plasmids in the anaerobic genus Bifidobacterium showed that only 4 of 24 species contained plasmids but revealed the existence of plasmid profiles in 3 of the species that may serve as molecular taxonomic markers for the identification of specific strains. (LeBlanc and Smith)

Administrative, Organizational and Other Changes

The Laboratory of Molecular Microbiology continues to play an important role in the training of young scientists. Dr. Kamal Chowdhury completed his postdoctoral fellowship with Dr. Mark Israel and joined the Department of Microbiology at Heidelberg University, Heidelberg, West Germany. Dr. Kaoru Segawa finished his appointment as a Visiting Fellow and returned to the Department of Virology, The Institute of Medical Science, The University of Tokyo.

In March, 1983 Dr. Mark Israel was appointed to a staff position in the Pediatric Oncology Branch, NCI. Dr. Joseph Bolen, an LMM Staff Fellow, accompanied him in this transfer. Dr. Yoshiaki Ito accepted an Expert position in the Laboratory of Molecular Oncology in August, 1983.

Dr. C. Jeffrey Smith was appointed as a Staff Fellow in January, 1983. He works with Dr. LeBlanc and studies drug-resistant plasmids present in indigenous, anaerobic bacteria. Ms. Laura Wilders joined the laboratory as a Clerk-Typist in May, 1983.

Honors and Awards

Dr. Malcolm Martin completed his tenure on the editorial board of the Journal of Biological Chemistry and continued to review papers for Science, Nature, Journal of Virology, and Virology. As an adjunct Associate Professor of Microbiology at the University of North Carolina, he supervised the dissertation research of Cecil Smith, who conducted risk assessment experiments at the NIH. Dr. Martin chaired the session on human retroviruses of the RNA Tumor Virus meeting held at Cold Spring Harbor, New York in May, 1983. In June, 1983 he was an Ad Hoc Reviewer on the Cell and Developmental Biology Study Section of the American Cancer Society. He continues to serve on the Recombinant DNA Executive Committee. During the year Dr. Martin presented lectures at the University of North Carolina, George Washington University, and Columbia University.

Dr. Donald LeBlanc as an adjunct professor, Ohio State University, supervises the dissertation research of masters degree candidate Larry R. Rollins, D.V.M. He is also a member of the Program Advisory Group for Contracts Related to the Use of Antibiotics in Animal Feeds, Division of Veterinary Medicine, FDA. He has presented lectures at the Universities of Toronto and Maryland.

Dr. Kenneth Takemoto was an invited speaker at the Conference on Acquired Immunodeficiency Syndrome and Kaposi's Sarcoma, held at Cold Spring Harbor Laboratory in February, 1983. He also delivered a lecture at the Division of Biology, Kansas State University, in November, 1982.

Dr. Joseph Tully is an Associate Editor, International Journal of Systemic Bacteriology, and Chairman, Subcommittee on Taxonomy of Mycoplasmas, American Society for Microbiology. He was the Chairman, Teaching Faculty and Course Director, International Mycoplasma Techniques course, University of Bordeaux, France, in the summer of 1983. During the year Dr. Tully gave lectures at the University of Alabama School of Medicine, the University of Southern Alabama School of Medicine, Ohio State University College of Medicine, the University of Bordeaux, and at the International Symposium on "Mycoplasma Hominis as a Human Pathogen" in Beito, Norway in March, 1983.

Dr. Arifa Khan was an invited speaker at the Department of Microbiology, George Washington University and the Tumor Virus Laboratory, the Salk Institute, San Diego, CA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00006-12 LMM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Competence Development and Genetic Exchange Mechanisms Among Streptococci

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Jon M. Ranhand, Sr. Scientist, LMM, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Bacterial Virulence

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Working within the interest of the laboratory, I initiated a study to determine why a specific antibiotic resistance (R) plasmid, pAM alpha 1, does not transform Streptococcus sanguis cells. This is a fundamental problem of gene function and gene control. pAM alpha 1 is a relatively small (6 MDal; 9 kb) plasmid that carries resistance to tetracycline (Tc). It was originally described in Streptococcus faecalis, strain DS5. A working hypothesis as to why pAM alpha 1 does not transform cells is that the plasmid, for some reason, either does not express Tc resistance or it cannot replicate in its new environment. To test these, I transformed cells with a hybrid plasmid that I constructed from the Tc resistant EcoRI restriction fragment from pAM alpha 1 and the replication-control genes contained on an EcoRI restriction fragment from pAM beta 1. pAM beta 1 is another plasmid originally described in S. faecalis DS5 cells that does replicate in S. sanguis cells. Since I have isolated a number of Tc resistant transformants, the hypothesis that Tc does not express has been ruled out. To date, I examined 6 out of 30 Tc-resistant clones. All contained plasmid DNA derived from pAM alpha 1 and pAM beta 1, as expected. This was demonstrated by standard Southern blot analysis using pAM alpha 1 and pAM beta 1 radioactive probes. The approximately 8 kb plasmid present in one strain of S. sanguis has been physically mapped. A surprising feature of the map is a loss of one EcoRI site. This loss permits the new plasmid, pJR4, to act as a potential S. sanguis cloning vehicle (not yet tested). The minimal inhibitory concentration for Tc in strains containing the hybrid ranged from 2.5 to 10 µg/ml and the resistance is constitutive. Control cells do not grow in 0.5 µg/ml Tc. Work will continue trying to establish the cause for the non-replication (non-establishment) of pAM alpha 1 in S. sanguis cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00013-20 LMM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Viral Antigens in Virus-Induced Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Andrew M. Lewis, Jr., Research Virologist, LMM, NIAID

COOPERATING UNITS (if any)

Arthur Levine, Cephas Patch, Heiner Westphal, NICHHD, NIH;
James L. Cook, National Jewish Hospital, Denver, Colorado

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Laboratory of Molecular Microbiology

SECTION

Viral Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.00

PROFESSIONAL:

1.00

OTHER:

2.00

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

DNA virus infections in humans are the suspected cause of significant morbidity and mortality from neoplastic diseases. DNA viruses also induce tumors in animals and transform normal cells to immortalized neoplastic cells in tissue culture. Transformed cells frequently have the capacity to produce tumors in animals. During transforming infections, portions of the viral genome become inheritable cellular elements; expression of specific regions of this genome is believed to induce the transformed state. The mechanisms by which transformed cells induce neoplasia are obscure. By studying the tumor-inducing capacity, the virus-specific antigenicity and the expression of susceptibility or resistance to lysis by cellular immune effector cells of lines of hamster cells transformed by adenovirus 2, adenovirus 12, SV40, polyoma virus, bovine papilloma virus and BK virus, we have developed a perception that, hopefully, reflects a basic feature of the conversion of normal cells to cells that produce tumors in animals. We are proposing that, during transformation, the virus conveys a specific level of susceptibility or resistance to immune rejection to the immortalized cell. The expression of this susceptible-resistant phenotype determines the success or failure of the cellular immune system of the potential host in eliminating the putatively neoplastic cell. Transformed cells that are susceptible to lysis by the immune defenses of the host will be eliminated, while transformed cells that are resistant to those defenses will present as clinically apparent neoplasias. The expression of the susceptible-resistant phenotype of transformed cells is an event that is independent of transformation per se and of the expression of the cell's virus-specific antigenicity. The reflection of the susceptible-resistant phenotype can be detected by in vitro assays and this parameter possibly represents the first in vitro assayable biologic feature of transformed cells that can be correlated with their tumor-inducing capacity. Using these concepts, we hope to be able to associate the induction of the transformed cell's susceptible-resistant phenotype with the expression of viral antigens and the function of viral proteins in transformed cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00018-17 LMM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological and Biochemical Characterization of Human Papovaviruses

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Kenneth K. Takemoto, Head, Viral Biology Section, LMM, NIAID

COOPERATING UNITS (if any)

Kunito Yoshiike, National Institute of Health, Tokyo, Japan

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Viral Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies on the biochemical and biological characterization of a new B-lymphotropic papovavirus (LPV) have continued during the past year. In biochemical studies of the viral genome, the origin of DNA replication was located. Using this as the reference point, we were able to align the genome of the virus to those of SV40 and BK virus based on data obtained by hybridization between specific fragments under low-stringency conditions. From the results of these experiments, the correlation between the physical and functional maps of the B-lymphotropic papovavirus genome was deduced.

We were able to demonstrate transforming capacity of LPV using hamster embryo cells. Two months after exposure to LPV, foci of transformed cells appeared and the cells could thereafter be passaged indefinitely. Preliminary studies on the analysis of the transformed cells have shown that the cells contain intranuclear T-antigens which show some cross-reactivity with SV40, BK, and JC T-antibody. Analysis of the status of the viral genome in the transformed cells has revealed that most, if not all, of the viral DNA exists in a non-integrated, free state. Immunoprecipitation of transformed cell extracts with anti-LPV serum showed that the large T-antigen of LPV is an 84K protein, the same as that previously found in infected cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00027-16 LMM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic Studies of Mycoplasmas

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Joseph G. Tully, Head, Mycoplasma Section, LMM, NIAID

COOPERATING UNITS (if any)

D. Taylor-Robinson, Clin. Res. Ctr., London; S. Razin & M.F. Barile, FDA;
J.M. Bove, Univ. Bordeaux, France; J.B. Baseman, Univ. Texas, San Antonio;
L.B. Senterfit, Cornell Univ., NY, G.J. McGarrity, Inst. Med. Res., Camden, NJ

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Laboratory of Molecular Microbiology

SECTION

Mycoplasma

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	1.0	2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

These efforts cover both basic and applied aspects of mycoplasmas and related wall-free prokaryotes, including their neurotoxins, antigens, biologic and genetic features involved in virulence, immunological interrelationships and possible role in human disease or diseases of uncertain etiology. Current work is directed to further characterization of a newly recovered mycoplasma (Mycoplasma genitalium) from urogenital tract of patients with non-gonococcal urethritis. The organism shares some partial antigenic relationships to another pathogenic mycoplasma (M. pneumoniae), and this relationship appears to be mediated by similarities in surface components of the unique terminal attachment structure found in both mycoplasmas. Recently, we have identified antibody to the major attachment protein (P1) of M. pneumoniae in the serum of patients with acute respiratory infections with this organisms. Experimental pathogenicity studies in a variety of sub-human primates indicates higher primates (chimpanzees) are a suitable model to measure ability of M. genitalium strains to colonize the male urethra, produce bacteremia and induce specific antibody responses. Experimental intravaginal challenge of female monkeys and marmosets indicated persistent colonization and inflammatory responses in the lower genital tract. Other investigations on urogenital mycoplasmas has shown that the use of SP-4 culture medium gave over 30% more isolations of M. hominis from human urogenital specimens than conventional mycoplasma media. This new medium, developed in our laboratory, has also been used to recover a new genital mycoplasma (Mycoplasma muris) from mice, the first new species from mice in over 30 years. A comparative study to assess genetic relatedness among established species of three genera of mollicutes (wall-free prokaryotes) has provided new support for current species distinctions, based primarily upon phenotypic markers. Organisms with strict host and tissue specificity (such as some Mycoplasma species) exhibited marked genotypic homogeneity, while those species recovered from a variety of hosts and habitats (such as some Acholeplasma species) are more heterogeneous in genetic characteristics.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00190-05 LMM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Molecular Genetics of Eukaryotic Cells and Their Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Malcolm A. Martin, Chief, LMM, NIAID

COOPERATING UNITS (if any)

George Khoury and Michael Kessel, LMV, NCI; Stephen O'Brien, LVC, NCI;
John Coligan and Lee Maloy, LIG, NIAID, NIH.

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Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

4.75

PROFESSIONAL:

2.75

OTHER:

2

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The principal aim of these investigations is the study of the molecular structure and function of endogenous type C retroviral DNA. Using techniques of molecular cloning, nucleic acid hybridization, and nucleotide sequencing, nearly 40 murine leukemia virus-related proviral DNA segments have been cloned from a human gene library. These retroviral sequences can be grouped into two classes: approximately 25% have the properties of a full-length type C retroviral DNA and contain LTR, gag, pol, and env regions. Nucleotide sequencing has revealed the presence of several characteristic features of retroviral LTRs including inverted repeats, several regulatory signals affecting transcription, and the presence of an adjacent polypurine tract (near the 3' LTR) and a putative t-RNA binding site (5' LTR). Extensive nucleotide and amino acid sequence conservation of the gag and pol regions of human and murine proviral DNAs was observed. The human env region has a large open reading frame with six potential glycosylation sites but shares no homology with the comparable region of any known retrovirus.

Normal and malignant human tissues have been screened for the presence of retrovirus-related RNAs using radiolabeled DNA probes derived from the LTR, gag, pol, and env segments of the cloned human endogenous retroviral DNAs. A tissue-specific pattern of expression has been observed employing RNAs prepared from human spleen, placenta, colon carcinoma, T-cell leukemia, retinoblastoma, and rhabdomyosarcoma cells, all of which contained human retroviral RNA as monitored by the dot-blot hybridization technique. Liver cells, B-cell lines, and several human carcinoma lines contained no detectable RNA. Northern blot analyses indicated that two different human placentas contained significant amounts of a 3.0 kb RNA species that anneals to LTR and env DNA probes. This reactive RNA, comigrated in a similar gel with the 21S spliced MuLV LTR-env RNA isolated from virus-producing cells. Additional RNA dot-blot and Northern analyses are in progress to establish the extent of endogenous retroviral expression in human cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00218-02 LMM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Chemical Studies on Retroviral DNA

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Roy Repaske, Research Chemist, LMM, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Additional clones of human genomic DNA shown to have murine leukemia virus (MuLV)-like reactivity were sequenced to determine the degree of nucleotide and deduced amino acid homology with MuLV in the structural protein (gag) and the reverse transcriptase (pol) regions. Approximately 4 kilobases of human DNA were sequenced. Computer analysis of these sequences showed regions of homology which were collinear with corresponding MuLV sequences. Intervening non-homologous sequences in human DNA corresponded in number to the MuLV sequence suggesting that the human MuLV-like sequence was complete. Typical highly conserved sequences among various leukemia viruses were identified in the gag proteins, p15, p30 and p10, as well as at the junction between p10 and pol.

Continuing studies on host range specificity of xenotropic MuLV focused on cloning an infectious xenotropic virus from the Hirt supernate of NZB infected mink cells. The infectious clone obtained gave positive immunofluorescence and S+L- complementation tests. In addition, the cell-free supernatant fluids from cells infected with cloned DNA were infectious.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00219-02 LMM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular and Genetic Analysis of Streptococci and Anaerobic Bacteria

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Donald J. LeBlanc, Acting Head, Bacterial Virulence Section, LMM, NIAID

COOPERATING UNITS (If any)

Drs. V. Scardovi & B. Sgorbati, Istituto di Microbiologia, Bologna, Italy

Dr. L. Rollins, Division of Veterinary Medicine, FDA, Rockville, MD

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Bacterial Virulence

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
4.75 2.75 2.00

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Conjugative plasmids that mediate resistance to antibiotics (R plasmids) among Streptococci generally fall into one of two categories. The large (50-80 kilobases [kb]), narrow host-range, R plasmids carry three or more resistance genes and transfer in broth culture, but only to members of a unique serological group. The small (22-30 kb) broad host-range, R plasmids code for only one or two resistance traits and can be transferred on membrane filters to virtually all species of Streptococcus. A restriction endonuclease map of the 80.7 kb narrow host-range Streptococcus faecalis plasmid, pJH1, was constructed and the locations on the map of the genes for resistance to kanamycin, streptomycin, tetracycline and erythromycin (Em) were determined. Similar plasmids isolated from S. faecalis strains of human and animal origin were found to share greater than 90% (human) and 50% (animal) DNA sequence homology with pJH1. The Em resistance gene of pJH1 and that of three human and three animal streptococcal isolates were located on the same transposable element, Tn917. The locations of the Em resistance gene, transfer region and replication functions were established on a previously constructed map of the 26.5 kb broad host-range plasmid, pAMB1. The results of cloning experiments permitted the localization of the replication origin and copy control region of pAMB within a 2.95 kb segment of DNA. A recent extension of our research program to the anaerobic genera, Bacteroides and Bifidobacteria, has revealed the presence of R plasmids coding for the same resistance phenotypes as those mediated by pJH1 and pAMB1. Cloned resistance genes from these two plasmids are being used for comparative studies with their counterparts on plasmids isolated from the anaerobic strains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00220-02 LMM

PERIOD COVERED

October 1, 1982 to August 1, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of the Oncogene Products of Polyomavirus

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Yoshiaki Ito, Visiting Scientist, LMM, NIAID

COOPERATING UNITS (if any)

Mark Willingham, Lab. Mol. Biol., NCI, NIH; Ettore Appella and Ken-ichi Tanaka, Lab. Cell Biol., NCI, NIH; Hiroyuki Shimatake, Lab. Biochem., NCI, NIH; and Tsuyoshi Kakefuda and Kimitoshi Kohno, Lab. Mol. Carcinogenesis, NCI, NIH.

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.2

PROFESSIONAL:

1.4

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We reported last year that tyrosine phosphorylation of middle T antigen appeared to be regulated by a cellular protein kinase(s). We have searched for such cellular enzyme and found that the epidermal growth factor (EGF) enhances tyrosine phosphorylation of middle T antigen. Since it is known that EGF receptor is a tyrosine kinase and that tyrosine phosphorylation of middle T antigen is considered to be essential for the induction of cellular transformation, it appears that extracellular mitogenic growth factors regulate the activity of tyrosine phosphorylation of middle T antigen and that mitogenic growth factors may play an essential role in inducing the transformation phenotype. We have constructed recombinant plasmids which express middle T antigen in *E. coli*. One of them have the amino-terminal polypeptide with 23 amino acids of λ phage O protein which is joined to the 21st amino acid of middle T antigen. This fused protein is not associated with a tyrosine kinase activity and it does not serve as an efficient phosphate acceptor when it is mixed with authentic middle T antigen. The results suggest that middle T antigen, at least as a primary translation product, is not itself a kinase. The antibodies directed against a nonapeptide, which represents a functionally important part of middle T antigen, label actin containing microfilament bundles of untransformed cells and immunoprecipitate 130K cellular protein. Possible significance of this crossreaction is under investigation. During characterization of mouse embryonic cell lines with respect to their permissivity for polyomavirus growth, we found an additional developmental stage, distinguishable from embryonal carcinoma cells and trophoblast cells, and which still supports viral replication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00222-02 LMM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Endogenous Ecotropic and Xenotropic Murine Leukemia Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Theodore S. Theodore, Research Microbiologist, LMM, NIAID

COOPERATING UNITS (if any)

Wallace Rowe, Janet Hartley, Charles Buckler, M. David Hoggan, and Herbert Morse, LVD, NIAID

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
1.25 1.25 0

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One model of leukemogenesis involves the generation of dual-tropic murine leukemia viruses (MuLVs) by molecular recombination between the spontaneously induced ecotropic MuLV and endogenous proviral DNA segments present in the chromosomal DNA of the mouse. Following the recombination event, a dual-tropic MuLV provirus may gain entry into a susceptible cell (such as a lymphocyte in the thymus) and, if integration occurs at an appropriate site (near a putative oncogene), disease may occur. Biological and genetic studies of inbred mouse strains indicate that endogenous ecotropic proviruses are differentially expressed in various strains of mice. AKR mice, which may harbor as many as five copies of such endogenous ecotropic proviral DNAs, begin producing virus shortly after birth, and 100% of animals develop leukemia within the first year of life. In contrast, BALB/c animals have only a single endogenous ecotropic provirus, sporadically produce low titers of ecotropic MuLVs, and only a minority (10 to 15%) develop disease. To evaluate why BALB/c animals inefficiently express ecotropic proviruses, we decided to molecularly clone the single copy of viral DNA present and compare it to the ecotropic proviruses found in AKR animals. Differences in the expression of infectious MuLVs could be due to alterations in viral structural or regulatory genes or might reflect the influence of flanking cellular DNA sequences.

During the past year, we utilized cosmid and λ phage vectors to isolate the ecotropic provirus from BALB/c chromosomal DNA. Five clones were isolated from a BALB/c library subsequent to the screening of 1.5×10^6 plaques. All five clones contained the 3' LTR and from 3.5 to 5 kb of pol and env retroviral sequences. Restriction mapping and blot hybridization techniques have verified an ecotropic proviral DNA has been cloned. The cloned segment is being transferred to a suitable plasmid vector prior to its assay in biological systems. The nucleotide sequence of the 3' LTR will be determined and compared with the previously published sequence for the highly infectious and readily inducible ecotropic proviral DNA isolated from AKR mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECTPROJECT NUMBER
Z01 AI 00353-01 LMM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology and Biochemical Structure of Endogenous Proviruses of Mice

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Arifa S. Khan, Senior Staff Fellow, LMM, NIAID

COOPERATING UNITS (if any)

Normal Salzman and Charles Rodi, LBV, NIAID, NIH; George Khoury, Peter Gruss, and Michael Kessel, LMV, NCI, NIH, Wallace P. Rowe and Joan Austin, LVD, NIAID, NIH; Bruce Chesebro, LPVD, NIAID, NIH, RML.

LAB/BRANCH

Laboratory of Molecular Microbiology

SECTION

Biochemical Virology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.0

PROFESSIONAL:

1.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

These studies are primarily directed towards understanding the expression of endogenous murine leukemia viral (MuLV) sequences in mice and their role in the generation of exogenous leukemogenic mink cell focus-forming (MCF) MuLVs. Endogenous MuLV DNA segments were molecularly cloned from the genome of BALB/c and AKR/J mice and extensively characterized by nucleic acid hybridization, nucleotide sequencing, and in DNA transfection experiments. The results are summarized as follows. 1) The long terminal repeats (LTRs) associated with a majority of the endogenous MuLV DNAs are distinct from the LTRs present in known exogenous MuLVs due to the presence of a 190 bp segment which possesses transposon-like features. This insert is located 48 bp upstream from the CCAAT box in the endogenous LTRs. 2) Nucleotide sequence comparison in the env region between an endogenous clone (A-12) and exogenous MCF247 MuLV DNA demonstrated that the two sequences were identical except for a few base changes. Comparison between the MCF and xenotropic MuLV sequences allowed identification of MCF unique nucleotides in env based upon which a 16 bp MCF-specific DNA probe was synthesized which did not react with xenotropic or ecotropic proviruses. 3) Recombinant MuLVs containing 3' env sequences from two different endogenous MuLV DNA clones encoded distinct p15^E envelope protein products.

The LTR associated with an endogenous African green monkey (AGM) retrovirus clone was also characterized. Nucleotide sequencing analysis indicated conservation of CCAAT and TATA regulatory signals and very little homology to the baboon endogenous viral LTR. The 3' AGM LTR demonstrated enhancer activity in an assay in which chloramphenicol acetyl transferase (CAT) gene expression was monitored.

LABORATORY OF PARASITIC DISEASES

1982 ANNUAL REPORT

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Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

SUMMARY - October 1, 1982 - September 30, 1983

INTRODUCTION

As this is written, LPD is gripped in the spasms of the renovations and moves that will bring the Malaria Section to Building 5. The annual ebb and flow of personnel was greater than ever and included the retirement of Ms. Wilma Paxton, who guarded the Lab Chief's door so effectively for many years--we wish her well! Our departing Visiting Fellows did unusually well in their research and appear well-equipped to continue strong performances with Andrew Simpson at Mill Hill (U.K.), Peter Gardiner at ILRAD (Kenya), Iroka Udeinya in Nigeria, Trisha Graves on a malaria project in Papua, New Guinea, and Vera Bongertz at FIOCRUZ in Brazil. Medical Staff Fellows: Terry Hadley takes a job at Walter Reed and Jim Leech leaves a bit later for University of California San Francisco--both still working on malaria. John Barnwell will start a new job with the Nussenzweigs at N.Y.U.. We hope that Juan Carlos and Patricia Engel, a gracious and hard-working pair, will be able to continue their work on return to Argentina. Several guest workers with whom we hope to keep in touch returned to their jobs, Drs. Correa-Coronas at the Navy and Carmen Tuazon at G.W. Medical Center.

Newcomers in the Visiting Program included Maria de Lourdes Munoz from Mexico to work with Eugene Weinbach, Ramesh Paranjape of India to work in Eric Ottesen's Lab, Dan Zilberstein (Israel) working with Dennis Dwyer and Nirbhay Kumar (India) with the malaria group. The latter two won special fellowships, Zilberstein a Weizman and Kumar a prestigious Burroughs-Wellcome award. David Lanar and Joanna Hansen are two new Staff Fellows, both molecular biologists, who will work on schistosomiasis and malaria, respectively. The new Medical Staff Fellows are James Sherwood from University of Rochester and Celia Maxwell from Howard University. In addition, we have a number of guest workers working in various areas. Drs. Ingelborg Perez of Mexico and Agneta Aust-Kettis of Sweden with Dr. Diamond, Drs. Antonio and Lucia Teixeira of Brazil are here to work on Chagas' disease. Dr. McNicol will be with the malaria group, and Victor Barbiero will shuttle between the LPD insectory and the Sudan.

Off-site field research included Dr. Ottesen's annual visit to Madras, India where he has developed an excellent collaborative program on filariasis and tropical pulmonary eosinophilia. Drs. Collins and Graves carried out an exciting study on a new way to identify malaria infected mosquitoes in the Gambia, West Africa, in collaboration with the British MRC Lab and a group from N.Y.U.. A follow-up visit on leishmaniasis cases in the Dominican Republic was made by Dr. Neva.

Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

SUMMARY - October 1, 1982 through September 30, 1983

HONORS AND AWARDS

Drs. Franklin Neva, Theodore Nash, Louis Miller, Alan Sher, Dennis Dwyer and Eric Ottesen served as consultants to the TDR program of the World Health Organization. Dr. Sher serves as Chairman of the Basic Science Steering Committee for schistosomiasis, Dr. Dwyer serves on the Steering Committee for Leishmaniasis and Dr. Miller on the Steering Committee on Immunology of Malaria. Dr. Ottesen is Chairman of the Scientific Working Group on Filariasis and serves on the Expert Committee on Filariasis and the Steering Committee on Onchocerciasis Chemotherapy.

Dr. Louis Miller received the Public Health Service Superior Service Award.

Dr. Alan Sher was the Block Chairman for Microbial Immunity for the annual meeting of the American Association of Immunologists.

Dr. Louis Diamond is President-Elect of the Society of Protozoologists, and Mr. Milford Lunde is President of the Helminthological Society of Washington.

Laboratory of Parasitic Diseases

National Institute of Allergy and Infectious Diseases

SUMMARY - October 1, 1982 through September 30, 1983

RESEARCH ACCOMPLISHMENTS

MALARIA IMMUNOCHEMISTRY,

Merozoite antigens and more about Duffy:

IMMUNOLOGY AND ENTOMOLOGY

Attention continues to be focused upon antigens of the merozoite because it is the stage of the malarial parasite that invades red cells. A

monoclonal antibody (Mab) has now been produced to a M_r 140,000 protein on merozoites which blocks invasion by agglutinating merozoites as they are released from the infected erythrocyte. But evidence for an even more specific type of immune reaction with merozoites has come from the demonstration that Fab fragments of IgG from monkey immune serum can react with the merozoite to block invasion (Miller, David and Hudson). Invasion of the red cell can also be blocked with Mab to a glycoprotein component of the membrane, called band 3 (Miller, Hudson and Hadley). With the technique of transfer of electrophoresed proteins onto nitrocellulose paper for staining with antibody (Western blot), specific staining of Duffy blood group antigen Fya has been demonstrated. The antigenic determinant identified has a size between M_r 35,000 to 40,000. This protein exhibits the same enzyme sensitivity as the determinant on the intact erythrocyte and can be split to a peptide that is still antigenically reactive (Hadley, Miller and David).

Surface membrane antigens of infected red cells: The only unequivocal example of antigenic variation among malaria parasites occurs with *P. knowlesi*. After a great deal of effort one of the variant antigens of this parasite has been identified as a high M_r protein, attached to the erythrocyte membrane, probably via interaction with the red cell cytoskeleton (Howard and Barnwell). Another very important erythrocyte membrane component, of even greater significance in human malaria, the histidine-rich protein associated with *P. falciparum* knobs, has been isolated (Leech and Howard). How this protein, which appears to be under the knob by electron microscopy, mediates attachment to endothelial cells to cause the small vessel pathology of falciparum malaria is still not known. The endothelial binding can be blocked by antiserum and shows specificity for different strains of *P. falciparum* (Udeinya, Leech and Miller). One of the long-standing problems associated with identification of merozoite antigens has been to sort out the changes in red-cell surface antigens that occur with schizont maturation and differentiate them from the antigens on the merozoite. A finding that will help in understanding this complex process is that a major schizont glycoprotein of M_r 230,000 appears to be processed into four small polypeptides by a proteolytic enzyme at the time of merozoite release (David, Hadley and Miller).

Antigens on sexual stages as targets for immune attack: The two monoclonal antibodies (Mabs) that block infectivity of *P. gallinaceum* for its mosquito vector were both shown to react with three proteins on the surface of male and female gametes (Kaushal, Rener and Carter). Analagous antigens of the

same approximate size have been identified by Mabs that block fertilization on gametes of *P. falciparum*, but some degree of strain specificity are exhibited by these antigens (Graves, Rener and Carter). The time sequence for synthesis of the target antigens in the *P. falciparum* gametocyte has been worked out, as well as a method to separate the high from the low molecular weight proteins (Kumar and Graves). Mabs have also been developed which identify two relatively small proteins (27 and 23K) on the surface of the zygote as it develops to the next sexual stage, the ookinete. Mab to one of these proteins will block development of the ookinete after fertilization has occurred (Kumar and Carter).

Studies related to the mosquito vector of malaria: For many years an important but time-consuming and tedious method to identify malaria-infected mosquitoes for epidemiologic studies has been the dissection of hundreds of mosquitoes. One by-product of the availability of monoclonal antibodies against sporozoites has been the utilization of such antibodies for a radioimmunoassay to detect, and identify by species, malaria sporozoites in infected mosquitoes. The test can be used on individual or pooled mosquitoes and can detect sporozoites resulting from even a single oocyst. A field study in the Gambia, in comparison with the hand dissection method, gave results indicating that the new immunoassay will be of great practical use (Gwadz, Collins, Graves and N.Y.U. collaborators). One theoretical approach to vector control of malaria would be to develop vectors that are resistant to the parasite in the hope that a resistant vector could compete in nature with one naturally susceptible. With this in mind, refractory lines of *Anopheles gambiae*, a highly efficient African vector, have been developed which are refractory to several species of *Plasmodium*, including an isolate of *P. falciparum* from Brazil. Interestingly, the refractory *A. gambiae* are only partially resistant to other isolates of *P. falciparum*. Linkage traits that accompany the refractory state are being sought to facilitate further genetic work (Collins).

OTHER INTRACELLULAR

PROTOZOA

Leishmanial parasite biology and immunology: For years parasitologists have puzzled over the apparent paradox that the culture-derived promastigote, which presumably initiates infection of macrophages, is easily killed *in vitro* by fresh normal serum and by macrophages. Is there some special infective form of leishmania, as is the case with trypanosomes? This question now seems to be answered by the finding that the virulence or infectivity of leishmania changes dramatically as the culture ages from log into stationery phase. The increased infectivity is both for macrophages *in vitro* and for the animal host, but is not associated with any obvious change in morphology (Sacks). Yet, it is unlikely that these findings can fully explain the wide spectrum of outcome when susceptible BALB/c mice are infected with different strains of leishmania. Optimum temperature for growth, for example, is one important factor that influences clinical course. This is exemplified by the fact that some varieties of cutaneous disease can be treated by local heat (Neva). Another determining factor, of course, is immune response to infection, which may be partly determined by characteristics of the parasite as well as by the host. Some isolates of leishmanial parasites are resistant to destruction by activated macrophages (Scott). Although B cells and/or antibody appear to play no role in immunity to cutaneous infection, they are required for immunosuppression to develop in a susceptible strain of mice. This was shown by μ -suppression (treatment with anti-mouse IgM) of BALB/c mice which then

exhibited greater resistance to infection (Sacks and Asfosky). Another bit of evidence for T-cell mediated immunity was shown by the ability of murine T-cell clones, stimulated by surface antigens of L. donovani to give passive local DTH responses for > 6 months in recipient mice, and to produce macrophage activating lymphokines in vitro. (Sheppard, Scott and Dwyer).

Leishmanial parasite membranes: Previous studies of the parasite membrane have emphasized chemical and antigenic structure. But now transport mechanisms by which specific substances move across this membrane are under investigation. A proton pump and specific [H⁺]-ATPase were identified in the surface of L. donovani, which drives sugar and amino acid transport. This may help explain how the organism can survive in the acid environment of the insect gut and within phagolysosomes of macrophages (Zilberstein and Dwyer). Identification of surface membrane components by external labeling continues. Most of these are glycoproteins containing mannose and/or galactose (Dwyer). Lipid analysis of L. donovani surface membranes has been completed (Wassef and Dwyer). The story of the extracellular acid phosphatase, originally found to be produced by strains of leishmania causing visceral disease, continues to unfold in a way that might provide taxonomic or clinical diagnostic utility. This enzyme is also produced by L. braziliensis, some Asian strains of L. tropica, but not by usual strains of L. tropica major or by the L. mexicana complex (Gottlieb and Dwyer).

Genetic diversity of T. cruzi: The investigation of both genotypic and phenotypic characteristics of the clones of T. cruzi has been continued and expanded. The findings from this work could account for much of the mystery that has surrounded the key issue of Chagas' disease - namely, why do relatively few of the infected individuals develop chronic disease, and why is there so much geographic variation in the disease? About 50 different clones of the parasite, derived from various sources including multiple clones from a single individual are available for study. Many clones are being examined in collaboration with other investigators for special characteristics, such as restriction endonuclease analysis (Morel in Brazil), drug sensitivity (Marr at Denver), metabolic and cell surface labeling (Dvorak and Howard) and reactivity to monoclonal antibodies (Kirchoff and Sher at NIH). One observation crucial to all these studies has already been established--namely, that the clones are stable, do not mutate and are easily cryopreserved (Dvorak). Several of the clones have consistently produced either an acute or a chronic infection in mice, the latter of which promises to be a useful experimental model of chronic Chagas' heart disease (Postan and Dvorak).

Antigen expression during development of T. cruzi: Additional characteristics were found of a 72,000 MU surface glycoprotein on the parasite that is identified by a monoclonal antibody (Mab). This surface epitope, which disappears as the parasite transforms to the tryomastigote form, was also found to disappear by simply placing the organisms at elevated temperature (Kirchoff and Sher). The overall implications and function of this particular antigen is less clearly understood by the finding that not all strains of parasite exhibit this epitope with the Mab probe. Further, both positive and negative clones were found within one strain (Kirchoff, Engel and Dvorak). Perhaps the important lesson here is that subtle changes in an epitope can determine reactivity with Mabs or a partial corollary of the old saying, "Believe nothing that you hear, and only half of what you see" - even with Mabs!

Bacterial product vs. T. cruzi: Work to further characterize the bacterial product with activity against T. cruzi is continuing. Purified fractions exhibit absorption spectrum at 216 nm, and the material is thought to be a small peptide.

MOLECULAR BIOLOGY
OF PARASITES

Plasmodial genes: One direction being pursued is to define the molecular basis for acquisition of drug resistance by malarial parasites. This would be very difficult to approach for chloroquine, since the mechanism of action of the drug is unknown, but the dihydrofolate reductase gene provides a "handle" to such an inquiry in the case of pyrimethamine resistance. This gene seems to be amplified, i.e., an actual increase in gene copies, in the parasite when resistance is acquired (McCutchan). The genes that code for the stable RNAs (ribosomal, transfer and 5.8S and 5S RNAs) have been characterized and mapped by DNA restriction analysis. Two of the ribosomal genes have been cloned and compared by heteroduplex analysis and S1 nuclease mapping. Based on these data the genes were found to have large differences in their primary sequences (Dame and McCutchan). A new screening technique has been developed for detection of antigenic materials from plasmodia, which is based upon expression of gene proteins in bacteria (McCutchan).

New tools to study Giardia: Two different applications of molecular biology have been made in regard to Giardia infections. Specific DNA probes were able to detect down to 500 organisms, but the utility of this technique for detection in the stool remains to be determined. Using Southern blot analysis of giardia DNA restricted with various endonucleases and hybridized to giardia DNA probes, several distinctive patterns were found in isolates from humans and in isolates from animals. These probes provide a powerful tool for epidemiological investigations (Nash).

HELMINTH

INFECTIONS

Immunology of filarial infections: Qualitative analysis of IgG and IgE antibodies to a spectrum of filarial antigens using immunoblotting and immunoprecipitation techniques are being pursued to identify stage and species specific antigens. Quantitative differences in IgE levels in patients with different manifestations of filariasis are more striking than the qualitative pattern of antigen-antibody reactions (Ottesen and Hussain). Therefore, attention has now turned to investigating the regulatory mechanisms involved in IgE synthesis. In contrast to normals and atopic patients, spontaneous in vitro production of IgE is high in patients with helminth infections (Nutman, Ottesen and Hussain). T-cell lines are being established from the cells of patients to isolate clones producing specific regulatory factors that affect IgE synthesis (Nutman and Volkman of LCI). Disproportionately high levels of IgG₄ have been found in filarial patients, so the possibility that synthesis of IgG₄ may be linked to that of IgE is being investigated (Ottesen and Hussain). A comprehensive immunologic analysis of events associated with the Mazzotti reaction is underway with collaborators in Ghana. This is the constellation of clinical signs and symptoms that occurs in patients with onchocerciasis after treatment with diethylcarbamazine (DEC). One of the dramatic changes is an early drop in serum complement (C3 and C5), followed by a tissue and blood eosinophilia with degranulation of eosinophiles and mast cells (Francis, Ottesen, Frank of LCI and Awadzi of Ghana).

Immunology and pathology of experimental schistosome infections: Even though there is a great deal of conflicting evidence regarding immunity to schistosome infections, clarification of the evidence may come from use of animal models that permit a sharper definition of the questions being asked. In this light we welcome the discovery that a mouse strain, P/N, fails to develop immunity after vaccination, whereas most mouse strains develop reasonable immunity (James and Sher). Genetic analysis of this defect is being worked out (Oliveira). Another development in this field is the belated recognition, which took a "back seat" to fascination with the more colorful eosinophil, that activated macrophages are efficient killers of schistosomules (James). The effect of maternal infection with *S. mansoni* on neonates which are subsequently exposed to the same infection was studied. Neonates born to infected mice exhibited an increased mortality which could not be explained by differences in worm burden or failure to receive adequate nursing (Correa-Coronas and Nash). The association of *Salmonella* bacteria with schistosome worms, a relationship that occurs in humans, was studied with *S. japonicum* in mice and *in vitro*. Although bacterial pili mediate *in vitro* adherence of bacteria to the worms, pili were not related to bacteremia in infected mice. *Salmonella* adhered better to male worms *in vitro*, but *in vivo* bacteria were associated primarily with female worms (Tuazon and Nash). Different strains of mice infected with *S. mansoni* exhibit up to 5-fold differences in hepatic fibrosis. This lesion, it should be recalled, is the primary manifestation of serious human disease produced by the parasite. When genetic analysis of this trait was attempted by crosses between high and low fibrosis strains, inheritance was found to be multigenic. However, in back crosses of F_1 progeny to high or low fibrosing parents, it was found that the size of the granuloma around eggs was dissociated from degree of hepatic fibrosis. Thus, granuloma size, commonly thought to be an indicator of liver damage is clearly not the only parameter of importance in evaluating pathogenesis of schistosomal disease. But mouse genetics is not enough! Two different strains of parasite produced dramatically different levels of resistance to re-infection in mice. Furthermore, this characteristic was inherited in a dominant fashion (Cheever).

Studies on strongyloidiasis: Many Southeast Asian refugees are being encountered in our out-patient clinic with *Strongyloides* infection. Virtually all are asymptomatic but are potentially at risk if immunosuppressed. Antibody levels by ELISA are being followed to establish the pattern of decline that follows after successful treatment (Neva).

LUMINAL	<u>Markers for virulence in amebae:</u> The agglutination of induced <i>E. histolytica</i> induced by Con A has been suggested to be a useful marker for virulence. A more detailed study of this phenomenon has shown Con A agglutination to be affected by temperature, composition of media and stage of growth cycle of the parasite. For individual axenic strains of amebae, maximum Con A binding was found to vary from mid- to late-log phase. Con A binding could not be correlated with virulence. Isoenzyme studies using esterases have proved useful in resolving a pathogenic zymodeme of <i>E. histolytica</i> (classification of Sargeant) into sub-groups (Daggett of ATCC and Diamond). Attempts to better define the axenic medium for growing amebae continue (Diamond).
PROTOZOA	

Metabolic studies with Giardia: An interesting sequel to the serum-free medium story for Giardia is the finding that cholesterol is an absolute requirement for the organism and vitamins D₂ and D₃ are powerful inhibitors. The inhibition presumably has its basis in D₂ and D₃ being structural analogs of cholesterol that compete for uptake (Weinbach). Calmodulin, a Ca++ binding protein has been isolated from Giardia and purified over 300-fold for molecular characterization (Munoz and Weinbach). The effects of tricyclic antidepressant drugs, imipramine and chlorimipramine, on mammalian mitochondria have been under study because they affect electron transport in a complex manner. But a serendipitous and unexpected discovery was the lethal effect of these drugs in micromolar amounts on protozoa such as G. lamblia and L. donovani. This finding is being investigated further (Weinbach and Zilberstein).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00094-24 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Lumen Dwelling Protozoa: Studies on Nutrition, Differentiation and Pathogenicity

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Louis S. Diamond, Head, Parasite Growth & Differentiation, LPD, NIAID

COOPERATING UNITS (if any)

P-M. Daggett, American Type Culture Collection, Rockville, MD

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Parasite Growth and Differentiation

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
5.3 2.7 2.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Axenic growth of Entamoeba histolytica in semi-defined TI-S-114 medium was enhanced by addition of nine amino acids, calcium, magnesium and glycogen. A low-density bovine lipoprotein containing cholesterol substituted for bovine serum in two undefined media used for axenic cultivation of this parasite. An in-depth study of Concanavalin A induced agglutination of E. histolytica showed agglutination to be modulated by temperature and composition of media, and was most pronounced during late log-phase growth. Con A agglutination could not be correlated with pathogenicity. Reversibility of the phenomenon is reported for the first time. A spontaneous, reversible agglutination of E. histolytica, not blocked by α D-methylmannoside, a Con A inhibitor is reported. Quantitative kinetic studies of ligand induced surface receptor redistribution using FITC labeled Con A showed peak binding capacity occurred during mid-log-phase growth in a pathogenic strain of ameba, and during late log-phase growth of a non-pathogenic strain. Amebae, after internalizing surface bound Con A, were not susceptible to further Con A agglutination. Esterases have proved useful for isoenzyme separation of E. histolytica isolates belonging to a single pathogenic zymodeme (Classification of Sargeaunt). Giardia intestinalis infections were induced in both intact and immune deficient newborn mice via intragastric inoculation through the abdominal wall. Infections were self-limiting. Chronic infections could not be induced.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00097-25 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological and Cytochemical Pathology of Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Teresa I. Mercado, Research Physiologist, LPD, NIAID

COOPERATING UNITS (if any)

Pathology Branch, NHLBI, Laboratory of Chemistry, NIADDK, Biological Testing and Reference Standards Branch, BOB

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies on the anti-trypanosomal factor (ATF) from Pseudomonas fluorescens revealed that the ultrastructure of Trypanosoma cruzi trypomastigotes was extensively damaged following treatment with the active fractions. Alterations were detected as early as 10 minutes after the initiation of treatment and were progressive as shown by the almost total destruction of the parasites after 10 hours. In vitro studies with cultured fibroblasts disclosed a slower penetration of the cultures by trypomastigotes which had been treated with the ATF. This effect was attributed largely to the decreased motility of the parasites produced by the ATF. Further studies on the chemical characterization of the ATF suggested that the active factor is structurally a hydrophobic acidic substance, possibly a small peptide with a blocked amino terminus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00098-27 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Mechanisms of Energy Metabolism in Mammalian and Parasitic Organisms

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Eugene C. Weinbach, Head, Physiology and Biochemistry, LPD, NIAID

COOPERATING UNITS (if any)

See next page

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of aerobic energy metabolism in Giardia lamblia have demonstrated a proton pump coupled to a respiration of the intact trophozoites. This provides an important approach to the study of energy metabolism in a eukaryote lacking mitochondria. Calmodulin, previously identified by radioimmunoassays in G. lamblia and Entamoeba histolytica, has been isolated from G. lamblia and purified 333-fold by isoelectric precipitation and affinity chromatography. Electrophoretic analyses of the purified protein disclosed a sharp band that co-migrated with standard calmodulin isolated from rat brain. Both G. lamblia and E. histolytica contain Ca^{2+} -activated ATPases, indicative of one possible role of calmodulin in regulating protozoan metabolism. Continued studies to improve the yield of G. lamblia in mass culture have led to the development of a serum-free medium. Cholesterol is an absolute requirement in this medium, and vitamins D₂ and D₃ are potent inhibitors of the parasite's growth. Current studies are underway to investigate the mechanism of this inhibition. It is likely because of their structural analogy to cholesterol the vitamins are competitively inhibiting cholesterol uptake by the parasites. Studies of mammalian mitochondria have shown that the tricyclic antidepressants, imipramine and chlorimipramine, have profound effects on energy metabolism. These drugs also affect the physiology of parasitic protozoa. Biochemical studies of cultured leukemia L1210 cells demonstrated that succinylacetone, a powerful inhibitor of heme-biosynthesis in other murine leukemia cells, retarded growth of L1210 cells in vitro, and inhibited their respiration by a heme-independent mechanism.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00099-13 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biophysical Parasitology

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

James A. Dvorak, Research Microbiologist, LPD, NIAID

COOPERATING UNITS (if any)

T. E. Hall, Applied Clinical Engineering Section, BEIB, DRS; C. M. Morel, FIOCRUZ, Rio de Janeiro, Brasil; M. A. Miles, London School of Trop. Med. & Hyg.; R. Hoff, Harvard School of Public Health; J. J. Marr, Univ. of Colorado

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
2.0 1.0 1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with studies of the genetic diversity of Trypanosoma cruzi and the implications of this diversity in the presentation and course of Chagas' disease. Emphasis has been placed recently on the consolidation, correlation and extension of studies of about 50 T. cruzi clones derived from various sources. The T. cruzi clones can be divided into 13 distinct subpopulations on the basis of restriction endonuclease (EcoRI) digests of their kinetoplast DNA. Some of the T. cruzi clones derived from isolates maintained in the laboratory for long periods of time prior to cloning (e.g. Y strain) have identical EcoRI digest profiles. However, one of five clones of Tulahuen strain (maintained in continuous passage for over 30 years prior to cloning) still demonstrates a restriction endonuclease profile that is different from the other four. A correlation was established between EcoRI digest profiles and total DNA/organism as determined by flow cytometry. Flow cytometry studies of total DNA/organism were extended to include analyses with propidium iodide. Results were identical to those obtained with mithramycin indicating that the difference in total DNA/organism between clones is not due to differences in G-C content alone. Metabolic labeling (³⁵S-methionine) and cell surface protein labeling (¹²⁵I-Iodogen) of selected clones demonstrate the existence of marked genotypic and phenotypic differences. The phenotypic differences can be correlated to differences in immunochemical reactivity through the use of monoclonal antibody reactivity (or lack of reactivity) of the various clones. The intracellular growth rates of amastigotes correlate to the extracellular rate of growth of epimastigotes in LIT medium. Consequently, the diversity in LIT growth rates of the clones is not due to "nutritional mutants" or culture system deficiencies. The growth of epimastigotes in LIT medium can be correlated to the sensitivity or resistance of the organisms to allopurinol and/or allopurinol ribonucleoside-5' monophosphate (compounds that are being considered for clinical trials in the treatment of Chagas' disease).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00102-09 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Disease Caused by Infection with Intracellular Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Franklin A. Neva, Chief, LPD, NIAID

COOPERATING UNITS (if any)

E. Petersen, University of Arizona, Tucson, AZ; H. Bogaert, Institute of Dermatology, Santa Domingo, Dominican Republic; J. Convit, Instituto Nacional de Dermatologia, Caracas, Venezuela

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.2

PROFESSIONAL:

0.7

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Additional leishmanial isolates from patients were tested for ability to produce lesions in BALB/c mice, and are being typed by monoclonal antibody and iso-enzyme analysis. Isolates of particular interest are (1) some from Venezuelan DCL patients, (2) cloned isolates from mouse spleen and footpad that can be examined for optimum temperatures of growth, and (3) ability of different species to produce late metastatic lesions in resistant mice, in a manner that could serve as a model for human muco-cutaneous leishmaniasis. Local heat treatment, with favorable results in a DCL case, but mixed results in regular cutaneous leishmaniasis, was tested in Venezuela. Sequential studies of the development of cutaneous leishmanial lesions in the mouse have been initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00103-16 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunological Studies on Toxoplasmosis and Other Parasitic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)
M.N. Lunde, Research Zoologist, LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.5

PROFESSIONAL:

1.1

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Sera from Clinical Center AIDS patients were monitored for antibodies to Toxoplasma gondii and Entamoeba histolytica and were found to be similar to those you would find in any group of non-AIDS patients. Toxoplasma was, however, found at autopsy from a patient who died from other complications of AIDS. Seven clones producing antibody to Brugia malayi have been isolated by monoclonal antibody and are awaiting characterization and purification. The methodology to enable identification of parasite antigen in immune complexes has been explored and although still under investigation early findings indicate that it should be possible to detect antigen within complexes even those of antibody excess.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00108-12 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Biology and Immunogenicity of Malaria Sporozoites

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Robert W. Gwadz, Research Entomologist, LPD, NIAID

COOPERATING UNITS (if any)

R. Nussenzweig, A. Cochrane, NYU School of Medicine, New York

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.7	0.8	1.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

An immunoradiometric assay (IRMA) has been developed and field tested in conjunction with scientists at NYU which can detect and identify malaria sporozoites in wild-caught mosquitoes. The test uses species-specific hybridoma- produced monoclonal antibodies and can quantify sporozoite levels resulting from a single oocyst. The IRMA should prove to be an important tool for studies of malaria epidemiology.

Circumsporozoite (CS) proteins have been shown to be the protective antigens of several species of malaria. The CS polypeptides of the various malaria species can be differentiated using monoclonal antibodies although these species specific polypeptides are structurally related and belong to a family of homologous proteins. A cDNA clone that expresses the CS antigen of Plasmodium knowlesi in E. coli has been developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00161-06 LPD
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunochemistry of Parasitic Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and Institute affiliation) Theodore E. Nash, Senior Scientist, LPD, NIAID		
COOPERATING UNITS (if any) C. Tuazon, George Washington University Medical Center; B. Unger, Johns Hopkins University Medical School; A. M. Deelder, State University of Leiden, The Netherlands		
LAB/BRANCH Laboratory of Parasitic Diseases		
SECTION Host Parasite Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.8	OTHER:
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>Studies continued with specific schistosome and <u>Giardia</u> antigens. The association of salmonella and adult schistosomes was studied. <u>In vitro</u>, salmonella were mixed with schistosomes, salmonella were associated to a greater degree with male schistosomes than female and pili+ organism was associated to a greater degree than pili- organisms. However, <u>in vivo</u>, salmonella were associated to a greater degree with female worms and pili+ and pili- organism adhered to the same degree. P.I.+ and P.I.- salmonella <u>in vivo</u> caused the same degree of mortality in <u>S. japonicum</u> infected mice.</p> <p>Mice born to <u>S. mansoni</u> infected mice which were subsequently infected showed a greater mortality than mice born to non-infected mice. Neonatal sensitization may be a factor in early mortality in schistosomiasis.</p> <p>Two specific E-S schistosome antigens were compared. Using radiolabeled PSAP, antibody to PSAP and various inhibitory fractions PSAP and CCA were found to be antigenically different.</p> <p>An ELISA test was developed which detected specific <u>Giardia</u> antigens in stools. In patients, the test was 95% sensitive and specific.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00162-07 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Cytology of Host-Parasite Interactions in Parasitic Protozoa

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Dennis M. Dwyer, Supervisory Microbiologist, LPD, NIAID

COOPERATING UNITS (if any)

Dept. of Immunology & Infectious Diseases, The Johns Hopkins University;
Dept. Biological Sciences, Univ. Cincinnati; Bur. Vet. Med., USFDA, DHHS

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Cell Biology & Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
5.0 3.5 1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cell biology, biochemistry and immunology of Leishmania and Trypanosoma are investigated as models of intra- and extracellular parasitism, respectively. As all interactions between host and parasite occur, at least temporarily, at the level of the parasite surface membrane, thus emphasis is placed on: 1) integrated structural, biochemical and antigenic characterization of the intact and isolated parasite surface membrane (SM) and parasite released products and 2) defining the mechanisms by which parasites survive and circumvent host-defense systems. To those ends, various studies are employed involving: Subcellular fractionation, fine structure labeling and localization, radiolabeling, electrophoresis and chromatography assays, immuno-binding and labeling assays and in vitro culture.

Using such techniques, the major SM components/antigens of Leishmania sp. and procyclic Trypanosoma rhodesiense were identified. The major SM antigens to which leishmaniasis patients make IgG responses were delineated. Total lipid composition of Leishmania SM was determined and 3 SM phospholipases identified. Five phosphomonoesterases were identified, partially characterized and localized cytochemically in the Leishmania SM. The SM origin and characterization of 7 Leishmania-released antigens was demonstrated.

Leishmania SM-antigen specific murine T-cell clones were generated which passively produced DTH responses in mice and macrophage-activating lymphokines in vitro. Monoclonal antibodies and C-DNA libraries were made for isolation of SM antigens and their genes. The kinetics and mechanisms of Leishmania SM sugar and amino acid transport were investigated. Both a SM-proton pump and a specific $[H^+]$ ATPase were demonstrated. The former providing the electro-chemical potential for SM transport in this organism. These results underscore the relevance of the surface membrane to parasite survival and the need for its biochemical and immunochemical characterization.

The goals of this project are to provide fundamental bases for understanding the mechanisms of parasite survival. 25-17

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00197-04 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immune Recognition in Filariasis and Other Helminth Infections

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Rabia Hussain, Senior Staff Fellow, LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.75	1.0	0.75

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major aim of this project is to characterize immunoglobulin responses in helminth infections (primarily filariasis and schistosomiasis) with emphasis on IgE production, regulation and modulation. Sensitive radioimmunoassays have been developed and utilized for quantitating IgG and IgE antibodies.

Qualitative characterization in terms of what antigens are being recognized in various clinical forms of the disease is being carried out to understand immune recognition and its implication in the pathogenesis and/or defense of the disease. These studies would in addition provide information about antigens with better specificity in immunodiagnosis or epidemiologic studies. Finally, in vitro production and regulation of IgE synthesis is also under investigation to better understand the control mechanisms of IgE production.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00208-03 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Isolation and Characterization of Plasmodial Genes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Thomas McCutchan, Senior Staff Fellow, LPD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
3.0 2.0 1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have undertaken projects aimed at the isolation and characterization of three types of plasmodial genes: 1) genes involved in the acquisition of drug resistance by the parasite 2) genes that code for antigenic surface protein of the parasite 3) genes that code for the stable RNAs (ribosomal RNA, 5.8S RNA, 5S RNA and transfer RNA). Characterization of both pyrimethamine sensitive and pyrimethamine resistant parasites indicates that amplification of the dihydrofolate reductase gene is involved in the acquisition of drug resistance. The genes for ribosomal RNA, 5.8S and 5S RNA have been characterized by DNA restriction analysis. There are only four ribosomal genes in plasmodium. When these are compared by E M techniques, they are found to have large differences in primary sequence. The translation of these genes could lead to the presence of varying types of ribosomes. Finally a new immunoscreening technique has been developed to isolate antigenic proteins from plasmodium.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00240-02 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Culture Physiology and Antigenic Analysis of Sexual Stages of Malaria Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Richard Carter, Visiting Scientist, LPD, NIAID

COOPERATING UNITS (if any)

See next page

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

5.20

PROFESSIONAL:

3.20

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have identified a set of three proteins present on the surface of male and female gametes of malaria parasites which are the targets of anti-gamete malaria transmission blocking monoclonal antibodies (Mabs). Analogous proteins have been identified in two species of malaria parasite Plasmodium gallinaceum in chickens, and Plasmodium falciparum from in vitro culture in human red blood cells. The 3 target antigens of gametes of P. falciparum were of apparent m.w. of 255, 59 and 53 kilodaltons; the target antigens of P. galinaceum were of similar apparent m.w. All 3 proteins in P. falciparum are synthesized early in gametocyte development and before the formation of the gametes themselves. Both parasite species suppression of infectivity was mediated by two distinct Mabs each of which, however, precipitated all three proteins from the gametes or gametocytes. In P. falciparum the two suppressive Mabs recognized distinct epitopes on the gamete antigens one of which showed antigenic variation between different isolates.

Following fertilization the zygotes begin to shed their previous surface antigens and two new surface proteins of 27 and 23 kilodaltons appear on the surface of zygotes transforming into ookinetes as shown in studies with P. gallinaceum. Using Mabs, the 23 K protein has been demonstrated to be a definitive target of post fertilization transmission blocking immunity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00241-02 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification of Receptors for Merozoite Invasion of Erythrocytes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Louis H. Miller, Head, Malaria Section, LPD, NIAID

COOPERATING UNITS (if any)

J. David Haynes, Walter Reed Army Institute of Research, Washington, D.C.;
J. Rener, Hazelton Laboratories, Vienna, Va.; M. Aikawa, Case Western Reserve
University, Cleveland, OH.

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
3.5 2.5 1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The merozoite interacts in a receptor specific manner with the erythrocyte surface and is the stage against which immunity may work to block invasion. Thus, merozoite surface components are of interest for their role in erythrocyte recognition and as antigens for induction of protective immunity. We are now studying the processing of these molecules during the ultimate stages of parasite development, their role in reception and as immunogens for induction of protective immunity. The major glycoprotein of the P. falciparum has also been shown to be localized to the schizont surface and not on the erythrocyte.

The Duffy blood group determinant involved in junction formation with P. knowlesi and P. vivax has been identified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00242-02 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological and Biochemical Studies of Antigens on Malaria-infected Red Cells

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Russell Howard, Expert Consultant, LPD, NIAID

COOPERATING UNITS (if any)

See Next Page

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.0	5.0	1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The biological functions and biochemical identity of new antigens on the surface membrane of malaria-infected erythrocytes are being characterized to determine their importance for parasite survival and potential value as immunological or chemotherapeutic targets. Plasmodium falciparum - infected erythrocytes express antigenic knobs on their surface which mediate attachment to venular endothelium, thereby preventing passage of mature infected cells through the spleen. We are attempting to identify the functional (ie. binding) components at these knobs plus knob structural components under and through the erythrocyte membrane. An *in vitro* model has been developed for the *in vivo* phenomenon of sequestration of mature parasitized erythrocytes.

P. falciparum-infected erythrocytes bind specifically to human umbilical and endothelial cells and to a line of human amelanotic melanoma cells via the surface knobs. Since immune sera are capable of blocking or reversing attachment of infected cells to endothelial or melanoma cell layers we are developing the antibody reagents to attempt to identify the biochemical nature of knob components responsible for binding.

Plasmodium knowlesi - infected erythrocytes express on surface variant antigen which changes during chronic infection. The parasites capacity to express different antigens allows it to evade variant-specific immune responses. This antigen has been identified in noncloned and cloned parasites and has been shown to be tightly associated with erythrocyte cytoskeletal components.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00244-02 LPD
PERIOD COVERED <u>October 1, 1982 to September 30, 1983</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Developmental Adaptations of <i>Trypanosoma cruzi</i> to the Vertebrate Immune System</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) F. Alan Sher, Head, Immunology and Cell Biology Section, LPD, NIAID		
COOPERATING UNITS (if any) D. Snary, Wellcome Research Laboratories, Kent, England		
LAB/BRANCH <u>Laboratory of Parasitic Diseases</u>		
SECTION <u>Immunology and Cell Biology Section</u>		
INSTITUTE AND LOCATION <u>NIAID, NIH, Bethesda, Maryland 20205</u>		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>In this project, we have been studying developmental adaptations of <u><i>Trypanosoma cruzi</i></u> to the vertebrate host and, in particular, surface membrane changes occurring during the morphogenesis of epimastigotes (vector stage) to trypomastigotes (vertebrate stage). Using a radioimmunoassay, we analyzed the loss occurring during this transformation of a carbohydrate epitope recognized by a monoclonal antibody (29.26). Over half of the loss took place while the parasites were still in the epimastigote stage. This decrease in antigenic activity could be mimicked by elevation of cultured epimastigotes from 26°C to 37°C. The remaining loss in the antigen binding activity occurred during the actual morphologic transformation of epimastigotes into trypomastigotes. Surface labeling studies confirmed that this loss in reactivity was due to the disappearance from the membrane of the entire 72,000 MW molecule recognized by 29.26 antibody.</p> <p>In a related study, strains and clones of <u><i>T. cruzi</i></u> were screened for their reactivity with 29.26 antibody. Approximately half of the isolates tested failed to react in the radioimmunoassay. Positive and negative clones were identified within one strain. Surface labeling studies have suggested that this divergence in the reactivity of epimastigotes with 29.26 results in the case of some isolates from membrane changes rendering the epitope cryptic, in the case of other isolates from structural changes in the 72K molecule, and in the case of one isolate from absence of the entire 72K molecule.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00246-01 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Studies of the Genome and Surface of Schistosoma mansoni

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

F. Alan Sher, Head, Immunology and Cell Biology Section, LPD, NIAID

COOPERATING UNITS (if any)

Biomedical Research Institute, Rockville, MD

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Temporarily inactive because of change of personnel

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00248-02 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiology and Genetics of Anopheles Mosquitoes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Frank H. Collins, Staff Fellow, LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Malaria Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.1	1.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Research has focused on determining the genetic and physiological basis of malaria refractoriness manifested by a selected strain of Anopheles gambiae. This mosquito strain exhibits the capacity to block sporogonic development of a wide range of Plasmodium species (including Plasmodium berghei, Plasmodium knowlesi, several isolates of Plasmodium cynomolgi, and certain isolates of Plasmodium falciparum). The block is manifested in all cases by the appearance of encapsulated oocysts on the midgut of infected mosquitoes. Preliminary data suggest that inheritance of refractoriness is multifactorial, with one locus responsible for most of the difference between refractory and susceptible lines. The allele for refractoriness at this locus is recessive. The expression of refractoriness is also highly correlated with a particular esterase isozyme. The refractory line is monomorphic for this esterase type and individual mosquitoes from the unselected, parent strain of A. gambiae which exhibit this isozyme have invariably been found refractory.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00251-02 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Studies on Schistosomiasis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

F. Alan Sher, Head, Immunology and Cell Biology, LPD, NIAID

COOPERATING UNITS (if any)

F. A. Lewis, Biomedical Research Institute, Rockville, MD

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Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.2

PROFESSIONAL:

2.5

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mechanisms of protective immunity were studied in mice resistant to challenge with Schistosoma mansoni as a result of vaccination with radiation attenuated cercariae.

Previous studies in our laboratory had indicated that vaccinated mice develop low levels of antibody and T cell responses which wane with time. We showed this year that challenge infection of previously vaccinated animals results in dramatic anamnestic B and T lymphocyte responses as measured by anti-larval antibody titer and lymphocyte proliferation. Thus, vaccination induces a potent immunologic memory which is recalled by challenge infection.

In a related study, vaccination was shown to result in the induction of T cells which upon in vitro exposure to schistosomulum antigens produce lymphokines capable of activating macrophages to kill these larvae. Indeed, these activated macrophages could be elicited in vivo by intraperitoneal injection of mice with schistosomula. The lymphokine mediating macrophage activation was characterized by Sephadex gel filtration as possessing an approximate molecular weight of 50,000 and was found to contain gamma interferon activity.

A mouse strain P/N was identified which fails to develop resistance to challenge as a result of vaccination. Analysis of vaccinated P/N mice revealed that they failed to produce normal levels of delayed type hypersensitivity to schistosomulum antigens, and were defective in both antigen induced lymphokine production and macrophage activation. Furthermore, these mice produced lower levels of IgM anti-larval antibodies than did mouse strains developing high levels of vaccine induced resistance (e.g. B/6). Preliminary results of genetic crosses between P/N and B/6 mice indicated that the defects in vaccine induced resistance and humoral and cell mediated immunity in P/N mice are inherited as recessive traits and that there is a correlation between resistance and antibody response in backcross animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00253-02 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Immunologic Responses to Filarial Infections

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Eric A. Ottesen, Senior Investigator, LPD, NIAID

COOPERATING UNITS (if any)

S. P. Tripathy, Tuberculosis Research Center, Madras, India;
K. V. Thiruvengadam, Medical College of Madras, India; R. G. Hamilton
Johns Hopkins Medical Center; F. Skvaril, University of Berne, Switzerland

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Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.65	1.75	.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to define the humoral and cellular immune responses that relate to immunopathology, protective immunity and immunodiagnosis of patients with filariasis.

Though qualitative differences do exist in the IgE antibody responses of patients with different clinical manifestations of filariasis, more impressive are the quantitative differences in IgE responses. Recent attention, therefore, has turned to regulatory mechanisms of IgE antibody production. Blood mononuclear cells from helminth infected patients spontaneously produce abundant IgE *in vitro* in contrast to those from normals or atopic individuals. Work is now aimed at defining T-cell regulatory factors that can affect IgE synthesis.

IgG antibodies that block immediate hypersensitivity responses to parasite antigen have been described, and their pathogenetic significance is under study. The possibility that they are restricted to a single IgG subclass has led to detailed analysis of the IgG antibody response. Filariasis patients have a significantly greater proportion of their total and parasite antigen specific IgG as IgG₄ (with less IgG₁) than normals. Furthermore, the greatest % of IgG₄ specific antibodies were found in microfilaremic and T.E. patients. The significance of these unusual findings is under study.

Use of qualitative techniques such as immunoblotting and immunoprecipitation has allowed visualization of antigens and allergens recognized by individual patients with filarial and other helminth infections. Attempts to define parasite specific antigens and to develop monoclonal antibodies to them for use in immunodiagnosis (ID) are in progress. Additional ID studies to detect circulatory antigens in parasitized individuals have resulted thus far in assays that can detect antigen in situations where antibody concentrations are relatively low; current efforts are directed toward modifying these techniques to detect antigens obscured because they are bound tightly in immune complexes.

25-27

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00254-02 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Studies of the Host Response in Onchocerciasis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)
E. A. Ottesen, Senior Investigator, LPD, NIAID

COOPERATING UNITS (if any)

See next page

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:
1.3PROFESSIONAL:
1.3OTHER:
0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to study the host response to onchocercal infection in order to understand the pathogenesis of clinical disease, the immune mechanisms important in the persistence of the parasite within the host and in protective immunity, and to develop improved immunodiagnostic techniques.

The initial phase has involved detailed clinical and laboratory assessment of the severe side effects (Mazzotti reaction) that accompany treatment of the infection and limit the potential of mass chemotherapy for onchocerciasis. Twenty-five patients were treated at a research center in Ghana and subjected to intensive immunologic and clinical evaluation during their Mazzotti reactions. Most prominent of the immunological changes was a dramatic fall of serum complement levels within 2 hours of initiating treatment and evidence for activation of immediate hypersensitivity immune mechanisms including evidence of eosinophil and mast cell degranulation both morphologic around microfilariae being killed in the skin as well as biochemically with rises in serum eosinophil granule protein concentrations and urinary histamine output. Correlation between individual clinical responses with these and other immunologic changes is currently underway. Also in progress is a study of the immunopathology of onchocercal eye lesions using ocular tissue and fluids removed from patients at the time of cataract surgery.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00255-02 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Immunologic Responses to Schistosome Infections

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Eric A. Ottesen, Senior Investigator, LPD, NIAID

COOPERATING UNITS (if any)

E. Ruiz-Tiben and R. A. Hiatt, San Juan Laboratories, Center for Disease Control; B. Doughty, University of Pennsylvania School of Medicine; M. Hofstetter, University of Michigan School of Medicine

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Laboratory of Parasitic Diseases

SECTION

Host Parasite Relations

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical studies have defined a form of limited immunosuppression in patients with chronic schistosomiasis that develops progressively after initial infection. This suppression is characterized by poor or absent lymphocyte proliferative responsiveness to parasite antigens and the progressive diminution of the granulomatous response schistosome eggs *in vitro*. These latter responses seem to be determined by the balance between OKT⁺ (helper) and OKT8⁺ (suppressor) T-lymphocytes in patients' peripheral blood monocular cells.

In contrast to this T-lymphocyte functional decline, the levels of total and parasite specific IgE antibodies actually increase as schistosome infections become chronic. Qualitative analyses of these IgE antibodies (as well as of IgG antibody) are currently under way in an effort to define allergens and antigens that are stage- and species-specific and that could be of value immunodiagnostically.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00256-02 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunology and Cell Biology of Cutaneous Leishmaniasis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

David Sacks, Staff Fellow, LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology and Cell Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.4

PROFESSIONAL:

1.1

OTHER:

.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have investigated the contribution of B-cells and antibodies to both the resistance and susceptibility to cutaneous leishmaniasis in mouse strains rendered B-cells depleted by treatment with goat anti-mouse IgM antisera from birth (μ -suppressed). These studies confirm that immunity to cutaneous disease in a normally resistant mouse strain (C3H/HeJ) is antibody independent, but that unexpectedly B-cells and/or antibodies are required for the evolution of suppressed delayed type hypersensitivity (DTH) and the consequent disease susceptibility of BALB/c mice. The data suggest that a B cell dependent T-cell which is critically involved in the suppressor pathway is absent in μ -suppressed mice.

We have also been studying the nature of Leishmania promastigotes which are responsible for the initiation of infection within the vertebrate host. Promastigotes which emerge from the fly must become adapted to a potentially hostile vertebrate environment. It is known that culture derived promastigotes which have generally been used for study are readily killed by the non-immune vertebrate host. They are destroyed by fresh normal sera and by normal resident macrophages. We have found, however, that the virulence of culture derived L. tropica promastigotes changes dramatically depending upon their age in culture. Whereas the promastigotes taken from logarithmic phase cultures are completely destroyed within normal mouse peritoneal macrophages *in vitro* and are relatively avirulent *in vivo*, as the growth of promastigotes approaches stationary phase, their virulence both *in vitro* and *in vivo* steadily increases. In addition, stationary forms have significantly increased resistance to complement. We suggest that these changes which are seen during growth in culture mimic developmental events which occur within the sandfly, and that it is these transitional developmental forms which are required to initiate infection in the vertebrate host.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00257-02 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunology of Strongyloidiasis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Franklin A. Neva, Chief, LPD, NIAID

COOPERATING UNITS (if any)

J. Cicmanec, Meloy Laboratories, Rockville, MD; R. Genta, Hahneman Medical School, Philadelphia, PA

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Laboratory of Parasitic Diseases

SECTION

Cell Biology and Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.2

PROFESSIONAL:

0.6

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Three different areas of work are being pursued with Strongyloides stercoralis. One area involves further evaluation of the ELISA test for serum antibodies to the parasite in patients. This test has already been found to be useful in diagnosis of infection, but is under investigation now primarily to follow the decline in antibody levels after treatment. The ELISA test thus may be useful in diagnosis of active infections, and to confirm cure after treatment. Another direction of study is the preparation and testing of skin test antigens prepared from Strongyloides larvae for evidence of immediate hypersensitivity in patients. The third area of research is the use of the Patas monkey as a model for hyperinfection with the human parasite, S. stercoralis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00258-02 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Models for Chagas' Disease Using T. cruzi Clones and Inbred Mice

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Miriam Postan, WHO Fellow, LPD, NIAID

COOPERATING UNITS (if any)

R. E. Patterson and Palmeri, S., National Heart, Lung and Blood Institute

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Laboratory of Parasitic Diseases

SECTION

Physiology and Biochemistry

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

T. cruzi clones and inbred mice are being used to develop experimental models for Chagas' disease. These studies demonstrate that parasite genetics plays a major role in the course and outcome of an experimental T. cruzi infection. In addition, they demonstrate that the parasite population obtained from chagasic patients are heterogenous. Dose-response studies show that the size of the parasite inoculum modulates the course of a T. cruzi infection but it does not change substantially its final outcome. In addition, the long-term stability of two T. cruzi clones was demonstrated by their pathogenicity for C3H mice following various in vitro manipulations of the parasites.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00347-01 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Schistosomal Hepatic Fibrosis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

A. W. Cheever Assistant Chief LPD, NIAID

COOPERATING UNITS (if any)

M. A. Dunn, Department of Gastroenterology, Walter Reed Army Institute of Research; D. A. Dean, Immunoparasitology Branch, Naval Medical Research Institute

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Laboratory of Parasitic Diseases

SECTION

Host-Parsite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	0.5	1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Hepatic fibrosis is examined in mice infected with schistosome species pathogenic for man. Mouse strains develop markedly different degrees of hepatic fibrosis following infection with S. mansoni. Among inbred strains, Nmri mice show maximal fibrosis and C57BL/6 mice minimal fibrosis. Inheritance was multigenic with incomplete dominance. Different mouse strains showed similar patterns of fibrosis whether infected with S. japonicum or S. mansoni. S. haematobium, which does not produce hepatic fibrosis in man, also induced marked fibrosis in the mouse liver.

NOTICE OF INTRAMURAL RESEARCH PROJECT

201 AI 00348-01 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunity in Murine Schistosomiasis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

A. W. Cheever, Assistant Chief, LPD, NIAID

COOPERATING UNITS (if any)

P. Stirewalt, F. Lewis, and C. Richards, Biomedical Research Institute, Rockville, MD

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.0

PROFESSIONAL:

0.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Substrains of *S. mansoni* selected for varying infectivity to vector snails have been tested for their ability to induce immunity in mice. Two strains derived from the same patient and designated PRT-3 and PRC-3 induced markedly different degrees of resistance to reinfection after a bisexual first infection. The F-1 cross between these strains also produced high resistance, comparable to that induced by the "immunogenic" PRT-3 strain. These two schistosome strains have been selected for two generations on the basis of their ability to induce resistance, without evident success thus far. Unisexual infections with some schistosome strains have induced considerable resistance to second infections, but the relative importance of worm strain and mouse strain in the induction of resistance are unknown.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00350-01 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DNA Analysis of Parasites

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Theodore E. Nash, Medical Officer, LPD, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Host-Parasite Relations Section:

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
0.7 0.7 0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Specific Giardia lamblia probes were used in order to differentiate isolates of Giardia lamblia. Analysis of DNA restricted with 2 endonucleases and 2 different probes was able to distinguish 3 different patterns of banding from 6 isolates of G. lamblia. This technique can be used to identify isolates and would be useful in epidemiology studies.

Giardia specific probes were developed and used to detect minute quantities of DNA present in small numbers of organisms in hopes of developing an assay for Giardia specific DNA in stool. Although 500 cultured organism or less could be detected on nitrocellulose, when mixed with stool the sensitivity of the assay was greatly decreased.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00351-01 LPD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Parasite and Host Factors Controlling the Pathogenesis of Leishmaniasis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Phillip A. Scott, Staff Fellow, LPD, NIAID

COOPERATING UNITS (if any)

Dr. James Howard, Wellcome Research Laboratories, Experimental Biology Division, London, England

LAB/BRANCH

Laboratory of Parasitic Diseases

SECTION

Immunology & Cell Biology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.2

PROFESSIONAL:

1

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cutaneous leishmaniasis can be either a spontaneously healing or chronic disease, depending upon the strain of the parasite and the immunological status of the host. Using murine models, we are investigating both parasite and host factors responsible for the variable pathogenesis observed in leishmanial infections. C57BL/6 or C3H mice heal infections with L. tropica, but fail to heal following infection with the Maria strain, a L. mexicana strain isolated from a mucocutaneous leishmaniasis patient. In vitro studies have shown that the Maria strain is resistant to activated macrophage killing, while L. tropica is susceptible. This resistance to killing was observed in macrophages activated by lymphokines obtained from either BCG, L. tropica-, or Maria strain infected mice, and was shown not to be due to parasite-induced inhibition of killing mechanisms, since Maria strain-infected, lymphokine-activated, macrophages exhibited tumoricidal and toxoplasmacidal activity similar to uninfected macrophages. However, we have found that during phagocytosis of Maria, macrophages show a significantly smaller respiratory burst than when engesting L. tropica. We are currently pursuing the possibility that this difference in the production of oxygen intermediates may be a major factor in the resistance of Maria to killing.

We are also examining host immunological factors which influence leishmaniasis using BALB/c mice. Although these mice are extremely susceptible to leishmanial infection, they show almost complete resistance following vaccination. We are comparing normal and vaccinated animals to determine what immune mechanisms are responsible for the protection seen in these mice. Although these mice do not show delayed hypersensitivity responses, peritoneal macrophages exhibited leishmanicidal activity when tested in an in vitro system. In addition, macrophages from infected mice lost the ability to respond to lymphokine, while macrophages from infected vaccinated mice did not. T lymphocytes from vaccinated mice respond to leishmanial antigens and continuous T cell lines have been established. 25-36

LABORATORY OF VIRAL DISEASES
1983 Annual Report
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PHS-NIH
SUMMARY REPORT

ANNUAL REPORT OF THE LABORATORY OF VIRAL DISEASES, NIAID
October 1, 1982 to September 30, 1983

Dr. Wallace P. Rowe
Chief, Laboratory of Viral Diseases

Dr. Janet W. Hartley
Acting Chief, Laboratory of Viral Diseases

The Laboratory of Viral Diseases suffered an inestimable loss in July when Dr. Wallace P. Rowe, Chief of the Laboratory, died following a long struggle with cancer. His inspiration and direction, powerful even during months of illness, led to a very productive year and to the consolidation of efforts to expand interaction among the major areas of expertise now represented in the laboratory, namely virology, immunology, molecular biology, and pathology. This amalgam of interests and experience has proved to be mutually stimulating as well as highly productive.

The primary focus of the laboratory effort remains in murine retrovirus studies. Areas of emphasis include genetic and mechanistic studies of a variety of genetically based control systems affecting murine leukemia virus infection and expression, and hematopoietic disease induction; viral pathogenesis studies, among whose results were the discovery and characterization of 2 new rapidly oncogenic defective viruses, one falling into the SFFV category and one into the MSV group; and chromosomal mapping studies, a major outcome being the localization of 5 of the retrovirus-related onc genes.

We have been fortunate in the continued maintenance and introduction of fruitful collaborative studies. These include work with Dr. Nancy Hopkins, MIT, on the viral genetics of leukemogenicity and the genetic basis of disease specificity, utilizing in vitro recombinants constructed from segments of molecularly cloned viruses; with Dr. J. Stimpfling, MRI, on the virology of a wide selection of B10 H2 congenic mouse strains; with the Laboratory of Molecular Microbiology in attempts to clone endogenous MCF and xenotropic virus-related sequences; and with a number of investigators on the chromosomal mapping of interferon structural genes, mouse mammary tumor virus proviruses, and retrovirus preferred integration sites, utilizing interspecific somatic cell hybrids and molecular hybridization techniques.

Dr. Gordon Wallace's program to evaluate diagnostic procedures for ectromelia virus infection and to conduct epidemiological studies has progressed well. An ectromelia ELISA test has been developed and demonstrated to be sensitive and specific. The limited efficacy of the current vaccination program has been revealed, and studies of sensitivity to infection and natural transmission in inbred laboratory mice are essentially complete. Dr. Wallace has left the program to accept a Departmental position but work is being carried on by his associate Dr. Mark Buller.

Demonstration of virus genetic control of disease specificity. In NFS mice Friend MuLV induces erythroleukemia while Moloney MuLV induces T cell lymphoma.

Pathogenicity testing of in vitro recombinant viruses constructed from fragments of molecular clones of these viruses has revealed that replacement in the Friend virus genome of sequences derived from the U3 region of Moloney virus long terminal repeat results in a virus population which induces almost exclusively T cell lymphomas. Thus transcriptional sequences in U3 may determine target cell specificity. (Rowe, Hartley, N. Hopkins)

Identification of a new Friend helper virus resistance gene. Studies of strain differences in sensitivity to Friend MuLV-induced erythroleukemia have revealed a new resistance gene, designated Fher, which acts at a step in leukemogenesis subsequent to Friend MuLV replication. Although the Rmcfr restriction is not involved, inhibition of generation of MCF viruses is correlated with resistance to erythroleukemia. Interestingly, erythroleukemia resistant mouse strains develop myeloid and lymphoid leukemias; the apparent lack of a role for MCF viruses suggests that cellular genes important in these diseases could be detected. (Silver, Fredrickson)

Linkage of Fv-4 gene to a new MuLV-related env sequence. Novel MuLV ecotropic envelope gene sequences have been identified in mouse cellular DNA in close association with the Friend virus resistance gene Fv-4, carried by certain wild mouse populations. The Fv-4r allele appears to act by coding for a cell surface MuLV related glycoprotein which interferes with exogenous infection. Since the restriction map of the sequences is unrelated to any known MuLV, Fv-4r may represent a unique mouse gene antigenically similar to ecotropic virus gp70, or may represent a previously undescribed proviral sequence. (Kozak, Buckler, Ikeda)

Isolation of a new rapidly transforming MuLV. In the course of studies of the pathogenesis of non-thymic hematopoietic neoplasms in NFS mice congenic for various ecotropic MuLV loci, using cloned MCF viruses recovered from various myeloid and B cell tumors, an unusual tumor of macrophage origin yielded a defective virus which induces rapidly fatal (2-3 weeks) erythroblastosis and endothelial cell proliferation and transforms fibroblast cell cultures. Biochemical characterization of the virus is in progress in order to determine whether this virus possesses a unique onc sequence. (Hartley, Fredrickson, Buckler)

Characterization of a new spleen focus-forming virus (SFFV). A defective MuLV causing rapid erythroleukemias was recovered from a lymphoma of a mouse infected with the wild mouse ecotropic MuLV, Cas-Br-M. The agent was biologically cloned and shown by both biological and molecular genetic criteria to differ from previous isolates of SFFVs. (Langdon, Buckler, Silver, Hartley, Morse)

Infection of mice with certain retroviruses results in deformed whiskers. Mice infected at birth with Friend, Moloney, and certain wild ecotropic and amphotropic viruses develop abnormalities of the vibrissae consisting of erratic curvature, shortening, and loss. Although endogenous and exogenous MCF viruses of AKR origin do not induce the effect, genetic evidence suggests that newly generated MCF type recombinant viruses may be involved in the pathogenesis of the whisker deformities. (Rowe)

Ecotropic virus induces a spectrum of hematopoietic tumors. The wild mouse ecotropic MuLV, Cas-Br-M, was found to be unique among ecotropic viruses in inducing a great variety of tumors in mice infected as neonates. The tumors

included erythro- and myelogenous leukemias, T and B cell lymphomas and a megakaryocytic leukemia. (Fredrickson, Langdon, Hartley, Morse)

Graying with age in mice is related to early expression of infectious MuLV. Black mice infected in utero or through the milk with MuLV were found to exhibit premature graying. Interruption of milk-borne virus transmission by foster nursing on virus-negative mothers yielded mice with normal coats whereas normally black mice fostered on virus-positive mothers turned gray by 3 to 5 months. (Morse, Yetter, Hartley)

Use of in vitro recombinant viruses to study leukemogenesis. Leukemogenic MCF viruses and their nonleukemogenic ecotropic parental viruses are distinguished by differences in nucleotide sequences in the 3' end of the genome, in the gp70, p15 and LTR regions. We have tested for oncogenicity several in vitro recombinant viruses constructed from restriction endonuclease fragments of AKR MCF 247 and AKV ecotropic virus reassorted in various combinations. Viruses with gp70, p15, and LTR derived from MCF 247 are highly leukemogenic, those with 2 elements moderately so, and those with only 1 very weak. (Hartley, Rowe, N. Hopkins)

Genetic mapping of a chromosomal locus required for MCF virus replication. Mouse/hamster somatic cell hybrids were used to show that the recombinant MCF viruses and their ecotropic virus progenitors require different mouse chromosomes for replication. Mouse chromosome 1 was found to carry the genetic information necessary for MCF virus replication; this gene, designated Rmc-1, most likely codes for a specific cell surface receptor. (Kozak)

Chromosomal location of mouse mammary tumor virus (MMTV) proviruses. The chromosomal distribution of MMTV proviruses represented in 3 unrelated mouse strains, NFS, GR, and DBA, was determined by restriction enzyme analysis. Linkage analysis using isoenzyme markers showed that one MMTV of DBA is on chromosome 7, and one, shared by all 3 strains is on chromosome 1, close to the xenotropic MuLV induction locus Bxv-1. (Kozak, V. Morris, R. Callahan). Two of the 3 BALB/c MMTV proviruses are closely linked to the immunoglobulin gene clusters on chromosomes 12 and 16. (Kozak, V. Morris, R. Callahan)

Wild mouse populations identified which lack Fv-1 restriction. Several populations of European, Asian, and/or North African wild mice lack the Fv-1 mediated genetic restriction of N- or B-tropic ecotropic MuLVs which characterizes laboratory mouse strains. Initial results of genetic studies with one wild mouse strain, M. praetextus, and inbred mice indicate that this phenotype is controlled by a new allele of the Fv-1 locus. (Kozak)

Five oncogenes mapped to mouse chromosomes. Analysis by Southern blotting of DNAs from mouse/hamster somatic cell hybrids for sequences homologous to the transforming genes of the acutely transforming retroviruses has identified chromosomal locations for H-ras and fes (chromosome 7), sis (chromosome 15), akt (chromosome 12) and raf (chromosome 6). (Kozak, Hoggan, S. Staal, U. Rapp)

Preferred retroviral integration sites mapped. By use of somatic cell hybrids and nonviral probes representing certain preferred chromosomal integration sites, one site has been localized to chromosome 7, and 3 to chromosome 15; both chromosomes are of special interest since they are known to contain oncogenes and chromosome 15 aberrations are found in many mouse neoplasms.

(Kozak, P. Tsichlis, G. Peters)

Structural genes for interferon mapped. Somatic cell hybrids have been used to map the structural genes of interferon to chromosomes 4, 12, and X. (Kozak, P. Pitha)

Analysis of endogenous xenotropic MuLV-reactive sequences in mouse DNA. By Southern blot hybridization techniques using a probe specific for xenotropic MuLV env sequences, 28 distinct HindIII restriction endonuclease fragments have been identified in BALB/c cell DNA. In tests of DNA prepared from mouse/hamster somatic cell hybrids 15 of these fragments have been correlated with the presence or absence of various mouse chromosomes and the Bxv-1 locus controlling xenotropic virus induction in 5 different mouse strains has been assigned to an 18 kb fragment at the distal end of chromosome 1. (Hoggan, Kozak)

Dissociation of severe lupus-like disease from polyclonal B cell activation and IL-2 deficiency. B cell activation and altered IL-2-production by T cells have been considered to be two of the major hallmarks for severe autoimmune disease in different strains. C3H mice homozygous for the lpr mutation exhibit both these parameters of immune dysfunction but do not develop renal disease or hemolytic anemia. (Davidson, Morse)

Natural transmission of ectromelia virus in inbred mice. Of 7 commonly used strains of inbred mice tested, C57BL/6 and AKR were found to be significantly more resistant to a virulent strain of ectromelia virus. Tests for excretion of virus in feces and transmission to cage mates indicate that the virus replicates in disease resistant mice and thus these animals can serve as an unrecognized source of infection. This finding is of particular importance since C57BL sublines are so widely used in immunology and virology programs. (Wallace, Buller)

Honors and Awards

Dr. Hilton Levy received an Inventor's Award given by the National Technical Information Service of the Department of Commerce for his invention of Nuclease Resistant Hydrophilic Complex of Polyriboinosinic Polyribocytidylic Acid.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00011-18 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of small DNA containing viruses belonging to the family Parvoviridae

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

M. David Hoaglan, Staff Scientist, LVD, NIAID

COOPERATING UNITS (if any)

K.I. Berns, Univ. Florida, College of Medicine, Gainesville, Fla.

F. Brent Johnson, Depart. Microbiology, Brigham Young Univ., Provo, Utah

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, Bethesda, MD

TOTAL MANYEARS: PROFESSIONAL: OTHER:

.05

.05

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Work has continued at a low level with occasional serological identification of new parvovirus isolates and the extension of various consultative services along with the provision of standardized virus and sera. A serological survey for the presence of parvovirus antibodies in AIDS patients has been initiated (see new project numbers Z01 AI 00376-01 LVD and Z01 AI 00377-01 LVD).

Adenovirus-associated virus (AAV)-carrier cell clones continue to provide the basis for a study of the integration state of AAV. Soon after cloning no free viral DNA can be detected in such carrier clones. However after 60-100 cell transfers small amounts of unintegrated viral DNA is found. It has been further shown that passage of carrier cells under the condition of low serum concentration eliminates the inducibility the AAV carrier cells even though the presence of AAV-DNA can be detected by standard hybridization techniques.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00020-08 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Treatment of Disease with the Interferon System

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Hilton B. Levy, Head, Molecular Virology Section, LVD, NIAID

COOPERATING UNITS (if any) R. Herberman, R. Smalley, M. Chirigos, K. Foon, NCI, B.R.M.P.; M. Droller, Johns Hopkins Hosp.: Drs. Muggia & Levin, N.Y.U. CA Cntr.; E. Borden, U. Wisconsin Med. School; J. Reed, Portsmouth Naval Hosp.; B. Lampkin, Childrens CA Testing Grp; A. Salazar, Walter Reed; D. McFarlin, NINCDS; W.K. Engel, U.S.C.

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Molecular Virology

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL MANYEARS:

1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The N.C.I. Biologic Response Modifier Program has undertaken poly ICLC as a drug to study intensively. In preclinical testing a variety of augmentary actions on several immune functions have been noted, some of which do not appear to be interferon mediated. A Phase I study of biological response modifications in humans is under way in two institutions--N.C.I. and the Naval Hospital in Portsmouth, VA. Upon completion a Phase II study is planned. In addition the N.C.I. has agreed to undertake the monitoring of all cancer protocols with poly ICLC.

Six clinical therapy protocols are currently ongoing, involving neuroblastoma, breast cancer, renal cell carcinoma, malignant melanoma, dysimmune peripheral paralytic neuropathies, and multiple sclerosis. There were no beneficial effects in neuroblastoma and breast cancer. There was a partial response in 2 of 5 patients with renal cell carcinoma. It is too early to evaluate, even in a preliminary way the malignant melanoma study. In 14 patients with peripheral neuropathy, there were at least 9 with moderate to marked improvement. In M.S., there are indications of positive effects, but it is too early to have much confidence in the results.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI-00135-09 LVD

PERIOD COVERED

October 1, 1982 - September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Properties of Immunoglobulin Secreting Cells

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Herbert C. Morse, III, Senior Investigator, LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Virology and Cellular Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

0.75

PROFESSIONAL:

0.50

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Lymphomas of the B lymphocyte lineage occur in high frequency as spontaneous neoplasms of NFS mice congenic for ectotropic murine leukemia virus induction loci of AKR and C58 mice. These lymphomas have been transplanted and grown *in vitro* clearly defining them as transformed cells. The cells are, histologically, lymphoblasts, follicular center cells, or immunoblasts and bear surface markers characteristic of normal B cells-sIg⁺, I-A⁺, Ly-5 (B220)⁺, ThB⁺, Lyb-2⁺, Ly-17⁺ - or pre-B cells-sIg⁻, I-A⁻, Ly-5 (B220⁻), ThB⁻, Lyb-2⁺, Ly-17⁺. Mice with these tumors should prove to be valuable models for studying B cell neoplasia in man.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI-00138-09 LVD
PERIOD COVERED <u>October 1, 1982 - September 30, 1983</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Viruses and the Immune Response</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <u>Herbert C. Morse, III, Senior Investigator, LVD, NIAID</u>		
COOPERATING UNITS (if any) <u>P. M. Hoffman, University of South Carolina, Charleston, South Carolina</u> <u>J. H. Stimplfing, McLaughlin Research Institute, Great Falls, Montana</u>		
LAB/BRANCH <u>Laboratory of Viral Diseases</u>		
SECTION <u>Virology and Cellular Immunology</u>		
INSTITUTE AND LOCATION <u>NIAID, NIH, Bethesda, MD 20205</u>		
TOTAL MANYEARS: <u>3.0</u>	PROFESSIONAL: <u>2.0</u>	OTHER: <u>1.0</u>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A major gene controlling expression of infectious <u>xenotropic murine leukemia viruses</u> (X-MuLV) was mapped to chromosome 17 in linkage with H-2K. The effect of this locus appears to be restricted to the X-MuLV induction locus of F/St mice, located on chromosome 1. B10 mice congenic for the H-2 haplotypes of F/St, P/J, SM/J and <u>Mus molossimus</u> express high levels of infectious ecotropic and mink cell focus-forming (MCF) MuLV as a result of intrauterine and milk borne transmission of B-tropic ecotropic MuLV. Exposure to high levels of virus early in life result in <u>premature graying</u> and germ-line reintegrations of B tropic MuLV.</p> <p>Cas-Br-M, an ecotropic MuLV obtained from wild mice was shown to be unique among ecotropic MuLV in that it induces a wide spectrum of hematopoietic neoplasms including B cell, T cell, myelogenous, and erythroleukemias. In addition, a Cas-Br-M lymphoma was found to contain a unique <u>spleen focus-forming virus</u>.</p>		
26-8		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI-00205-03 LVD

PERIOD COVERED

October 1, 1982 - September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Abnormalities of T and B Lymphocytes of Autoimmune Mice

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation) Wendy F. Davidson, Visiting Scientist, LVD, NIAID and Herbert C. Morse, III, Senior Investigator, LVD, NIAID

COOPERATING UNITS (if any)

J. Roths and E. Murphy, The Jackson Laboratory, Bar Harbor, ME

R. Coffman, DNA Research Institute, Palo Alto, CA

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Virology and Cellular Immunology

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mice bearing the autosomal recessive mutation lpr were studied for abnormalities of hematopoietic cells related to the autoimmune disease produced by this gene. C3H mice bearing this mutation were found to have marked expansion of an abnormal population of cells with surface characteristics of both T cells and B cells. As immunoglobulin heavy-chain genes were not reorganized in these cells, the lpr gene results in a population of aberrant T cells that express B lineage antigens. SJL mice homozygous for lpr die of severe autoimmune disease and express markedly increased levels of infectious ecotropic murine leukemia viruses whereas heterozygous lpr mice express lower levels of virus and die with accelerated B cell tumors. However, continuous in vitro cell lines established for spleens and lymph nodes of homozygous or heterozygous mice have yielded granulocytic tumors.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00280-02

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ecotropic murine leukemia virus proviral sequences in inbred mice.

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Charles E. Buckler, Research Biologist, LVD, NIAID

COOPERATING UNITS (if any)

M. A. Martin, LMM, NIAID

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL MANYEARS: PROFESSIONAL: OTHER:
.25 .25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Fv-4 gene of mice confers resistance to infection by MuLV. This dominant locus which maps to chromosome 12 has been found to be associated with DNA sequences that hybridize to an ecotropic MuLV env-specific probe. A physical map of the portion of the mouse genome that contains the Fv-4 related ecotropic env sequences has been determined. The relative locations of 12 restriction sites in a region of 23 kilobases of mouse genome that surround the Fv-4 MuLV env related sequence were obtained. The restriction map is unlike that of any known MuLV and thus the Fv-4 locus represents either a novel provirus or a fragment of a MuLV provirus containing only env related sequences. The physical map obtained will aid in the design of experiments to clone the segment of the mouse genome that contains the Fv-4 locus.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00281-02 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular cloning of recombinant MuLV proviral DNA sequences from AKR tumors

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Charles E. Buckler, Research Biologist, LVD, NIAID

COOPERATING UNITS (if any)

T. Theodore and M.A. Martin, LMM, NIAID

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL MANYEARS:

.75

PROFESSIONAL:

.75

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The molecular cloning of recombinant MuLV proviral sequences present in thymomas of AKR mice is in progress. DNAs prepared from thymomas induced by the inoculation of 19 AKR/J neonates with MCF-247 viruses were analyzed by Southern blot hybridization. The tumors contain multiple copies of recombinant MuLV provirus DNA sequences in addition to the endogenous MuLV-related sequences present in the AKR/J genome. A DNA library has been constructed from one tumor and a recombinant proviral sequence has been isolated from the library. Molecular subclones from the isolated recombinant containing flanking mouse cellular DNA sequences will be utilized to examine insertion sites in other tumors. Additional libraries from other tumors will also be constructed to evaluate whether common integration regions play a role in the pathology of MuLV induced tumors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00282-01 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Malignant Lymphomas Occurring in Congenic Mice

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

T.N. Fredrickson, Research Microbiologist, LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The above project was inactive during the period October 1, 1982 to September 30, 1983.

DPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SLRVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00283-01 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Pathogenesis of Chronic Myelogenous Leukemia in NFS/N Mice

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

T. N. Fredrickson, Research Microbiologist, LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The above project was inactive during the period October 1, 1982 to September 30, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00284-02 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of pathogenic murine leukemia viruses.

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Janet W. Hartley, Research Microbiologist, LVD, NIAID

COOPERATING UNITS (if any)

Nancy Hopkins, MIT

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SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL MANYEARS: PROFESSIONAL: OTHER:

2.0

.75

1.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The virological factors, both biological and molecular, affecting the pathogenicity of murine C-type viruses of the ecotropic and recombinant MCF virus classes are under study. Differences in nucleotide sequences in the env and LTR regions distinguish leukemogenic AKR MCF viruses from their non-leukemogenic AKV ecotropic progenitor. In vitro recombinants have been constructed in which restriction enzyme segments of molecularly cloned AKV and MCF viruses were exchanged and rearranged. Oncogenicity testing of the resultant viruses indicates that gp70, p15E, and LTR sequences all contribute to some degree to leukogenicity. Tests of in vitro recombinants between molecularly cloned Friend and Moloney MuLV indicate that control signals in the LTR region play a key role in determining virus-target cell specificity. During a study of the pathogenicity of viruses derived from wild mice, a new rapidly transforming defective virus has been isolated: biological and molecular studies are in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00286-02 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tissue culture studies of genetic resistance to murine leukemia viruses

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

Janet W. Hartley, Research Microbiologist, LVD, NIAID

COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL MANYEARS: PROFESSIONAL: OTHER:
0.7 .2 .5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A host resistance gene has been identified in mice which specifically suppresses in vitro and in vivo replication of the MCF class of murine leukemia viruses. Designated Rmcf, the gene is dominant for resistance, is located on chromosome 5 very close to the locus for hammer-toe (Hm), and is carried by DBA/1, DBA/2 and certain sublines of CBA (i.e., those related to CBA/H). The biological importance of this virus resistance gene is being explored in pathogenesis experiments, including tests of mouse strains partially congenic for Rmcfr.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00287-02 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chromosome mapping of endogenous xenotropic MuLV proviral DNA in inbred mice.

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

M. David Hoggen, Staff Scientist, LVD, NIAID

COOPERATING UNITS (if any)

M.A. Martin (LMM, NIAID)

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Viral Oncology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL MANYEARS:
1.10PROFESSIONAL:
.40

OTHER:

.70

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Mirrors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have now examined over 30 somatic cell hybrid clones from Chinese hamster and various mouse cells for the presence of endogenous xenotropic env related DNA sequences. It has been possible to correlate 15 of 28 xenotropic related HindIII restriction fragments from the BALB/c mouse with the presence or absence of various chromosomes. Of importance is the finding that the inducible Bxv-1 locus found in 5 different mouse strains can be assigned to an 18 kb fragment located at the distal end on chromosome 1 along with 2 other fragments (13 and 9 kb in size). A 10 kb fragment is located at the other end of chromosome 1. Of 11 other identifiable fragments, one each can be assigned to chromosomes 2,4,5, 12,16,17 and 19 while 2 each are located on chromosomes 7 and 15. Detailed studies have further shown that the majority of the xenotropic reactive fragments have internal restriction maps comparable to either known xenotropic proviruses or MCF precursor viral sequences. As part of this project we have used the same somatic cell hybrids to map the mouse homologues of various oncogenes. These include C-Harvey ras and C-fes to chromosome 7 and C-sis is to chromosome 15.

Our long range goal is to study all xenotropic related sequences in the mouse with the aim of clarifying their role in oncogenesis.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00288-02 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular cloning of endogenous xenotropic MuLV proviral DNA

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

M. David Hoggan, Staff Scientist, LVD, NIAID

COOPERATING UNITS (if any)

M.A. Martin and T. Theodore (LMM, NIAID)

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Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL MANYEARS: PROFESSIONAL: OTHER:

.35

.20

.15

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ongoing studies of the organization and expression of endogenous xenotropic MuLV DNA sequences have highlighted the need for cloning each of the 28 defined HindIII restriction fragments found in BALB/c mice. The availability of such a clone library would allow us to better define the role in virus expression repression and tumor induction of each proviral MuLV sequence along with its flanking DNA. We used the cosmid system to begin our cloning attempts. Preliminary data suggested we had cloned a number of xenotropic proviral DNA sequences including the most prominent 11.6 kb fragment. This fragment is not only found in BALB/c mice but is common to most mice tested. However every attempt to amplify the various primary cosmid clones resulted in all xenotropic env reactive insert fragments being lost. We then tried cloning the various BALB/c HindIII restriction fragments in the Charon 20 vector. The efficiency of this vector was too low and we were again unable to successfully clone any appropriate fragment. We have now initiated studies using the λJ-1 vector. Thus far, HindIII arms which can be shown to be easily ligated have been prepared as well as concentrated fractions of appropriately sized HindIII restriction fragments from somatic cell hybrid DNA. Attempts to isolate and characterize appropriate clones continues.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00289-02 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Organization of endogenous proviral DNAs of xenotropic murine leukemia viruses.

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

M. David Hoggen, Staff Scientist, LVD, NIAID

COOPERATING UNITS (if any)

M.A. Martin (LMM, NIAID)

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National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL MANYEARS: PROFESSIONAL: OTHER:
.30 .15 .15

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Restriction endonuclease analysis of genomic DNA from various inbred and wild mouse strains have been carried out using the Southern blotting technique and a xenotropic reactive recombinant DNA probe. Except for a few distantly related mus species, the DNA from all mice tested contained many copies of xenotropic proviral DNA. Of these 18-50 copies in each strain at least 4 appear to be common to all inbred mice and the remainder have been used to identify characteristic patterns of each strain which can be used to define genetic relatedness between them. As previously reported our studies of internal organization have allowed us to define at least 7 related but distinct families of xenotropic endogenous sequences in inbred mice. Using these patterns we hope to analyse the DNA from various tumors to see if any particular fragments show polymorphism compared to standard liver DNA. Attempts to clone specific identifiable sequences will continue. To determine why some of these endogenous sequences are expressed as xenotropic virus, as MCF recombinant virus or not all in some mouse strains but not others, continues as the primary long range goal of this project.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00299-02 LVD

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic control and mapping of endogenous proviruses

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Christine Kozak, Senior Staff Fellow, LVD, NIAID

COOPERATING UNITS (if any)

Dr. Vincent Morris (Univ. Western Ontario); Drs. Robert Callahan and Michael Potter (NCI)

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National Institute of Allergy and Infectious Diseases

TOTAL MANYEARS: PROFESSIONAL: OTHER:

.82

.41

.41

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Investigation of the genetic transmission of germline copies of murine retroviral genomes has led to the chromosomal mapping of more than 10 distinct loci. Studies with related laboratory mouse strains and Asian mice have been undertaken to describe the stability and wild mouse origin of specific loci and to identify additional genetic factors which alter *in vivo* virus expression and susceptibility to exogenous virus. Most recently, mendelian crosses are being analyzed to describe the genetic basis for the great differences in induction efficiency shown by the apparently allelic ecotropic loci of DBA and SEA mice. Studies on the transmission of the endogenous mouse mammary tumor virus (MMTV) in sexual crosses have resulted in the characterization of the complete and fragmented proviral genomes carried by several inbred strains. One full-length MMTV provirus was chromosomally localized near the MuLV locus, Bxv-1. Finally, studies with feral mouse populations have shown that many lack germline copies of ecotropic and xenotropic retroviruses. Cells of wild mice also differ from inbred mice in their susceptibility to exogenous infection, and genetic crosses are being used to analyze these differences. Preliminary studies have identified a new allele of Fv-1 in *M. praetextus*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00300-02 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic control of resistance to Friend virus in inbred wild mouse populations

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Christine Kozak, Senior Staff Fellow, LVD, NIAID

COOPERATING UNITS (if any)

M. Potter, NCI

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INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases

TOTAL MANYEARS:

.83

PROFESSIONAL:

.42

OTHER:

.41

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Various wild mice are resistant to induction of erythroblastosis by Friend complex virus. The Japanese mouse, M. m. molossinus, carries a locus apparently identical to the Friend virus resistance gene, Fv-4, described originally in G strain mice. Sensitivity to Friend complex disease was examined in these mice to characterize Fv-4, and identify any other genetic factors which influence virus replication and the development of disease. Southern blot hybridization has shown that a novel ecotropic envelope-related sequence is integrated at or near the Fv-4 locus. Infectious ecotropic virus cannot be induced from mice carrying only Fv-4 associated sequences, but immunological methods have identified a unique cell surface antigen on the thymocytes of Fv-4 resistant mice which shows the serological reactivity of an ecotropic envelope glycoprotein. The restriction map of this sequence is different from that of other ecotropic proviruses, and this sequence is now being molecularly cloned for further analysis. A number of other feral mice are also resistant to Friend complex disease, and several of these mice also carry this Fv-4 associated sequence. However, the resistance shown by M. spretus more closely resembles that shown by Fv-2 resistant mice, and results of crosses with Fv-2 sensitive and resistant inbred mice suggest that M. spretus carries the resistance allele at this locus.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00301-02 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Somatic cell genetic studies on endogenous proviruses

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Christine Kozak, Senior Staff Fellow, LVD, NIAID

COOPERATING UNITS (if any)

S. Staal and P. Pitha (Johns Hopkins)

R. Callahan, P. Tsichlis, D. Nebert, U. Rapp, S. O'Brien (NCI)

G. Peters (ICRF, London)

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TOTAL MANYEARS: PROFESSIONAL: OTHER:

1.2

.6

.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The use of interspecific hamster/mouse somatic cell hybrids has greatly extended our studies on the genetic basis of tumorigenesis. We have used hybrids to identify and chromosomally map specific genes involved in oncogenesis and to construct cell lines carrying isolated genetic elements of this complex multi-gene system for their further characterization. Recent studies have mapped receptor loci for the ectropic and MCF viruses to different mouse chromosomes. Hybrids which lack specific receptor loci were used to describe the time course of virus inducibility from different endogenous proviral loci. Receptor loci for different leukemia viruses are also being identified and mapped in other species using cat/hamster and human/hamster hybrids. Finally, somatic cell hybrids are being analyzed by blot hybridization with molecularly cloned probes to describe the chromosomal localization of proviral and other cancer-related loci. These studies have resulted in the localization of MMTV proviral loci to 3 mouse chromosomes and xenotropic envelope-reactive sequences to almost all of the mouse chromosomes. Analysis of hybrids has provided specific map locations for various proto-oncogenes, for interferon structural genes, and for 4 loci representing preferred integration sites for retroviruses in tumors.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00302-02 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Congenic mice with genes of importance to murine leukemia virus infection

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Wallace P. Rowe, Chief, LVD, NIAID

COOPERATING UNITS (if any)

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National Institute of Allergy and Infectious Diseases

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	.5	1.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A number of genes are being bred onto standard inbred mouse backgrounds, chiefly the NFS Swiss. These genes include ecotropic virus-inducing loci, virus-resistance genes, and linkage marker genes for chromosomal regions of particular interest. The V-loci congenics have permitted the initial demonstration that the virus-inducing loci were in fact viral genomes, have allowed characterization of the genetic control of their induction, have allowed mapping of some, have allowed biochemical identification of the proviruses from multi-copy high-virus strains, have allowed identification of the crucial importance of virus titer in early life for rate of subsequent leukemia and the general independence of leukemia experience on the source and chromosomal location of the endogenous provirus, have shown the crucial importance of maternal antibody in determining the life long viral phenotype, and have demonstrated the occurrence of germline reinsertions of viral genomes. Together, the congenics permit an unprecedented degree of genetic analysis and strain development for analysis of viral leukemogenesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00303-02 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic resistance to murine leukemia viruses and virus-induced neoplasms.

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Wallace P. Rowe, Chief, LVD, NIAID

COOPERATING UNITS (if any)

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National Institute of Allergy and Infectious Diseases

TOTAL MANYEARS: PROFESSIONAL: OTHER:

1.7

.7

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The genetic basis of tumorigenic responses to endogenous or inoculated C-type viruses is being studied in a variety of strain crosses. The major determinants of spontaneous leukemias in hybrids with AKR have been shown to be the endogenous ecotropic virus inducing loci, the Fv-1 gene, and the Rmcf gene. The progeny of crosses of AKR with certain strains of mice (C57L, C57Br, C57BL/6.Fv-1ⁿ, SJL) which are Fv-1ⁿ and, based on in vitro assays, Rmcfs, show restriction of MCF generation and low frequency of thymic lymphoma. The basis for this type of restriction is under study. The Rmcf gene, which specifically inhibits infectivity of MCF viruses, is a strong determinant of leukemogenicity by MCF 247 and Friend helper virus. The effect of this gene on a variety of retroviral diseases is under study, including spontaneous B-cell lymphomas in high virus congenic mice, inoculated with erythroblastic or myeloid leukemia-inducing viruses, and mice inoculated with a neuroparalytic retrovirus. No clear effect of the restrictive Rmcf allele on Moloney, Gross, or SL3-3 virus-induced T cell lymphomagenesis has been observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 AI 00304-02 LVD
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Hematologic and genetic studies of malignancies induced by Friend helper virus		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Jonathan Silver, Medical Staff Fellow, LVD, NIAID		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Viral Diseases		
SECTION Viral Oncology Section		
INSTITUTE AND LOCATION NIAID, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: .75	PROFESSIONAL: .75	OTHER:
CHECK APPROPRIATE BOX(ES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)		
<p>Friend helper virus induces a variety of hematopoietic neoplasms including erythroblastosis, myeloid leukemia, and T and B cell lymphomas. These neoplasms are clonal and contain Friend helper virus integrated into cellular DNA. Analysis of mouse strain differences in the type of leukemia induced by Friend helper virus has led to identification of a new Friend virus resistance gene designated <i>Fhe^r</i> which makes C57BL mice resistant to Friend helper erythroblastosis. This gene acts at a step subsequent to Friend helper replication. Friend helper virus does not induce recombinant mink cell focus-forming viruses in C57BL/6 mice as it does in mice susceptible to Friend helper erythroblastosis. This finding opens up the possibility that cloning Friend helper virus-cell DNA junction fragments in C57BL myeloid leukemias and lymphomas will lead to the identification of unique cellular sequences which play a role in these tumors. AKR mice are also resistant to Friend helper virus erythroblastosis but develop accelerated thymic lymphomas after Friend helper virus inoculation. Genetic studies to identify AKR genes involved in resistance to Friend helper virus erythroblastosis are in progress.</p>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00305-02 LVD

PERIOD COVERED

October 1, 1983 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Mechanism of Fv-1^b Inhibition of AKR Thymomagenesis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Jonathan Silver, Medical Staff Fellow, LVD, NIADDK

COOPERATING UNITS (if any)

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Viral Oncology Section

INSTITUTE AND LOCATION

National Institute of Allergy & Infectious Diseases, Bethesda, MD

TOTAL MANYEARS: PROFESSIONAL: OTHER:
.10 .10

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Congenic AKR.Fv-1^b mice express about 10 to 50-fold less ecotropic virus in the thymus than AKR mice, and do not develop thymic lymphomas at 6 to 12 months of age like their AKR counterparts. To study whether the Fv-1^b effect is mediated by thymocytes or thymic stroma, reciprocal subcutaneous thymus graft and bone marrow chimeras were constructed between AKR/Cum (Fv-1ⁿ, Thy1.1) and AKR.Fv-1^b (Thy1.2) mice. Thy1 isotypes were used to determine the genotype of thymocytes in chimeric mice. In thymic graft chimeras and young bone marrow chimeras, the Fv-1 type of thymocytes rather than thymic stroma determined the amount of ecotropic virus expressed by thymocytes. In older bone marrow chimeras, high amounts of ecotropic virus were expressed by thymocytes independent of Fv-1 type. Attempts to repeat and confirm these results have been temporarily delayed by failure of the AKR.Fv-1^b mice to breed.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00306-02 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Biology of Mousepox

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation) R.M.L. Buller, Visiting Associate, LVD, NIAID and G.D. Wallace, Senior Investigator, LVD, NIAID

COOPERATING UNITS (if any)

H.C. Morse, III, LVD, NIAID; Dr. Moss, LBV, NIAID; Dr. Weinblatt, ATCC; Dr. Potter, LCBGY, NCI; Dr. D. Taylor, Georgetown University

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Viral Oncology Section

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National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL MANYEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ectromelia virus, an orthopoxvirus that can cause extensive morbidity (mousepox) in colonized mice, has been epizootically responsible for serious disruptions of biomedical research since 1930. The last outbreak of mousepox at NIH, which began in 1979, was estimated to have cost more than one million dollars in surveillance and control efforts. The cost in thwarted and delayed experiments cannot be estimated. The goals of this proposal were to: (1) develop a sensitive and specific serologic assay using the principles of the enzyme-linked immuno-absorbent assay (ELISA); (2) study the kinetics of natural transmission (including clinical response) among commonly used strains of inbred mice, such as BALB/cByJ, C57BL/6J, and DBA/2J; (3) measure the efficacy of immunity induced by the IHD-T strain of vaccinia virus in BALB/cByJ mice; (4) evaluate the risk of virus transmission through mouse-derived tumors (hybridomas) and sera; (5) undertake preliminary studies on the genetics of innate resistance in outbred (Mus cervicolor popaeus, Mus platyrrhix, Mus pahari, Mus spretus, Mus musculus, and Mus domesticus) and inbred mouse populations (C57BL/6J, BALB/cByJ, A/J, DBA/2J); (6) study the epizootiology of naturally occurring outbreaks of mousepox, and (7) correlate various ectromelia biological strain (strains: Beijing 78, Ishibashi, St. Louis, Wash. U., Moscow) differences to DNA genome sequence which will aid in the studies of epizootics.

Of the above mentioned goals, the first five have been completed and are either in press or in various stages of preparation for publication. It is anticipated that the remaining two goals will be finished in 1983-1984 fiscal year. The basic studies and diagnostic methodology described above are essential for a rational approach to the control and prevention of mousepox, which threatens serious disruption of biomedical research in the U.S.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00375-01 LVD

PERIOD COVERED

October 1, 1982 - September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Diagnosing of Murine Hematopathology

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Torgny N. Fredrickson, D.V.M., Ph.D., LVD, NIAID and University of Connecticut

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.15	.15	

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Independently made diagnoses of neoplasms, induced by Cas Br M virus, by morphology and identification of cell surface antigens were remarkably nondordant. These results prove the value of the morpho-physiologic approach in diagnosis of neoplasms of the murine hematopoietic system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00376-01 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Serum survey of parvovirus antibody in normal and at risk AIDS populations

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

M. David Hoggan, Staff Scientist, LVD, NIAID

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL MANYEARS:

.25

PROFESSIONAL:

.25

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although the immune deficiency syndrome (AIDS) is characterized by the appearance of severe often fatal opportunistic infections and epidemiological studies suggest that some virus like microorganism is involved in the genesis of the condition no agent has as yet been incriminated in the etiology of the disease. Because of the pathology seen in a number of known parvovirus infections, such as feline panleukopenia, neonatal mouse infection with immunosuppressive strains of minute virus of mice (MVM), and human infection with the B19 virus, recently shown to be responsible for aplastic crises and erythema infectiosum in children, as well as the timely appearance of a new fatal virus disease in dogs caused by a parvovirus, these agents need to be examined in great detail. The specific goal of this project is to determine the distribution of antibody and/or antigen to known parvoviruses in control as well as at risk for AIDS populations using standard serological techniques already developed within this laboratory.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00377-01 LVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Survey of SAIDS tissues and fluids for parvovirus antigens and antibody

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

M. David Hogan, Staff Scientist, LVD, NIAID

COOPERATING UNITS (if any)

Murray Gardner, Regional Primate Center, Davis, CA

LAB/BRANCH

Laboratory of Viral Diseases

SECTION

Viral Oncology Section

INSTITUTE AND LOCATION

National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD

TOTAL MANYEARS:

.20

PROFESSIONAL:

.20

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Serious outbreaks of a disease which is similar to the acquired immune deficiency syndrome in man have occurred in the Rhesus monkey populations of 2 regional primate centers in the U.S. The stricken animals become subject to many opportunistic infections including CMV and the disease has recently been transmitted to normal animals using cell free filtrates. Even though numerous agents have been studied, no specific etiological agent has as yet been proven to cause the disease. One group of agents that have not been investigated as possible agents in the geneses of the disease are the parvoviruses. It is the purpose of this project to analyse material serially collected from selected animals for the presence of parvovirus antigens and/or antibody. In the second phase of this study we plan to study various tissues of infected animals for the presence of parvovirus DNA using standard hybridization techniques.

LABORATORY OF MICROBIAL STRUCTURE AND FUNCTION
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Annual Report
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RESEARCH HIGHLIGHTS

Major emphasis in LMSF is on structural and functional aspects of bacterial surface components that bear on the organisms' interactions with human and/or animal hosts. Diverse bacterial pathogens are studied; they include obligate intracellular parasites, exclusively extracellular organisms, and some with "mixed" intra- and extracellular life styles. In spite of their diversity in these regards, several of the bacteria studied are being probed with similar/identical methodologies; the answers that are forthcoming have both common and unique themes. One common theme concerns expression of structurally different forms of a given surface component. Gonococci and borreliae exhibit a startling array of structural permutations for outer membrane proteins. Such an array clearly "confuses" or "outstrips" the host's immune system as it allows the organisms to display a seemingly continuously changing surface facade. This diversity contrasts with the extremely conserved, constant surface constitutions of rickettsiae and some bordetellae. Little difference was found also among Lyme disease spirochetes isolated from various European and U.S. sites.

For most of the bacterial systems studied in LMSF, the past year involved continued descriptive biochemical and immunochemical endeavors aimed at defining those portions or forms of particular outer membrane components which are biologically active. These data aim for elucidation of primary structure of outer membrane proteins and their active infra peptides; this information is being utilized in construction of mixed-polynucleotide probes needed for both molecular cloning of relevant genes and understanding genetic mechanisms involved in expression of a particular surface component. A few of the more noteworthy findings produced by the past year's endeavors are summarized as follows:

Gonococcal surface components: Pili are thought to serve as anchors of gonococci (Gc) to human hosts' mucosal surfaces by virtue of the adhesive properties of the pilus-appendages; interruption of Gc adherence by anti-pilus antibodies elicited by immunization with pili is the rationale of a vaccine presently being tested. During the past year, it has been demonstrated that a given strain of Gc can exhibit 12 different structural forms of pili: changes in pilus subunit structure occur *in vitro* at very high frequency (0.01 - 0.1% of a single colony's progeny); a change in pilus subunit size is accompanied by a change in colony morphotype, and this finding greatly simplifies definition of pilus variants among Gc grown on solid medium *in vitro*. Preliminary ¹²⁵I-peptide mapping suggests that different subunit forms of pili may have quite similar primary structures and differ only in a single alpha-chymotryptic peptide. These findings of high frequency mutability in Gc pilus structure strongly suggest that formulation of a pilus vaccine which would include the necessary immunizing array of pilus subunit forms will be a difficult task. Preliminary experiments to define genetic mechanisms responsible for changes (gain/loss) in pilus expression and for changes in pilus subunit

form are underway; rearrangements in Gc chromosomal DNA are found in several forms attendant to changes in piliation. Current results suggest a complex arrangement of three pilus structural genes in the homologous strains' genome; two of the three appear as constant sized features of the strains' chromosomal DNA, but the third exhibits variation in size (Cla I digestion) in both piliated and nonpiliated organisms (Swanson and Barrera). A very recent finding is that pili of two different diameters reside on Gc of the same strain (Todd and Swanson). The relationships between these apparently very different kinds of pili with their subunit size, immunochemical, and cell-adhesion properties will be examined.

Gc outer membrane lipopolysaccharide (LPS) has been previously shown to occur in a few (3-6) varieties; probably all these forms have endotoxic properties. Recent studies have shown that there is far more structural heterogeneity for Gc LPS than defined in earlier studies. Development of a silver stain which seems specific for LPS separated by acrylamide gel electrophoresis and use of proteinase K digestion of detergent solubilized bacteria allow rapid and reliable portrayal of an organism's LPS. Methods were also devised for immunoblotting analysis of LPS antigens with poly- and monoclonal antibodies. These studies have documented over a dozen different profiles of Gc LPS molecules. All are semi-rough by analogy with Salmonella chemotypes; both common and variable antigens are found among the various LPS species resolved electrophoretically (Hitchcock).

Studies on outer membrane protein I have led to realization that previously-described "hairpin" orientation in the Gc outer membrane may only relate to certain subunit forms of protein I. Recent studies using proteinase K digestion of whole Gc show that some protein I molecules display one end (not the center "hairpin" bend) on the organism's surface. This form of protein I is found in Gc that cause disseminated Gc infections and are serum-resistant. The different orientation of these organisms' protein I molecules likely correlates to their resistance to killing by pooled human sera and may also relate to the disease spectrum they cause (Swanson and Barrera).

Gc gene banks have been established in several plasmid and phage vectors. Recently, monoclonal reagents have been developed to react with several different outer membrane protein II species. Further, an oligonucleotide probe (constructed from partial sequence of protein I) has been made, and synthesis of pilus gene-like oligonucleotides is nearly complete. These reagents, along with development of a new cloning vector, are expected to allow fine genetic analysis of encoding of these Gc surface proteins during the coming year (Mayer).

Chlamydial surface components: The major outer membrane proteins (MOMP) of chlamydiae were shown to be major serotyping antigens of these organisms, and a sensitive radioimmunoassay for detection of chlamydiae in clinical specimens was devised. Polyclonal antibodies monospecific for a particular form of MOMP neutralizes infectivity of homologous serotype chlamydiae in tissue culture cells. More recently, monoclonal antibodies directed against MOMP were found to also neutralize the homologous serotype organisms' infectivity; this monoclonal antibody recognizes a serotype-specific epitope whereas a species-reactive monoclonal (also against MOMP, but a different epitope) did not neutralize chlamydial infectivity. Monoclonal antibody directed toward an LPS-like molecule of the chlamydial outer membrane did not

neutralize infectivity; this anti-LPS monoclonal reacts with all members of the genus. Cyanogen bromide fragmentation of MOMP and immunoblotting revealed a 9K dalton fragment as bearing the serotype-specific epitope (Caldwell). This fragment is being sequenced as is the N-terminal portion of a purified MOMP (Barstad) for defining the structure of an exposed portion of MOMP and for constructing a mixed oligonucleotide probe for studying genetic mechanisms responsible for MOMP expression.

Other studies on chlamydial MOMP disclosed marked differences in structures of elementary bodies (infectious form of chlamydia) versus reticulate bodies (intracellular multiplication form); intra-MOMP disulfide linkages are found for MOMP of elementary bodies whereas available cysteine groups (not cross-linked into disulfide bridges) were demonstrable in MOPMs of reticulate bodies. These data define an intracellular (in host cells) change in organization of MOMP that likely relates to the organisms multiplication within host cells' phagosomes. Another fascinating finding was that proteolytic cleavage of MOMP on whole, intact chlamydiae did not alter their infectious abilities. This was unexpected because of the role of MOMP in infection which was suggested by infectivity neutralization with anti-MOMP antibodies noted above. At present, it is not clear whether cleavage of MOMP can be "repaired" intracellularly (in host cell) or whether an intact MOMP subunit is irrelevant to chlamydial intracellular infections (Hackstadt).

Surface components of spotted fever rickettsiae: Considerable progress was made toward defining surface-exposed constituents of Rickettsia rickettsii. Strains of differing virulence and differing isolation locales were compared in their outer membrane protein and LPS components. Remarkable conservation occurred among these strains for nearly all outer membrane components; but two differences were found for the very low virulence versus high virulence rickettsiae. Monoclonal reagents that recognize rickettsial LPS were developed and these were used along with antisera to define the major antigens of the rickettsial surface by several immunochemical assays including immunoprecipitation and immunoblotting. LPS species among spotted fever rickettsiae were defined by methods just recently developed in LMSF. In all these assessments, only subtle differences in surface constituents of different spotted fever rickettsiae were defined (Anacker).

Surface components of Borrelia hermsii and of the spirochete causing Lyme disease: In collaboration with investigators at Yale University College of Medicine and the New York State Department of Health, the spirochete previously isolated at RML from ticks and implicated as causing Lyme disease was isolated from patients with this disorder. These demonstrations complete establishment of the Ixodes tick-related spirochete as the etiological agent of Lyme disease (called *erythema chronicum migrans* in Europe). The identities of the clinical isolates were established by a variety of immunochemical and gel electrophoretic methods previously developed for characterizing the tick-isolated spirochetes. This spirochete bears some antigenic relations to relapsing fever borreliae, but unlike these borreliae, the Lyme disease spirochete displays little or no strain-to-strain variability in its outer membrane components even when isolates from different sites in Europe and the U.S. are compared. In contrast, the relapsing fever borreliae exhibit an array of rapidly changeable serotype-specific proteins; progressive, sequential expression of different serotype-specific proteins by borreliae correlates with immunologic eradication of one serotype and "relapse" with a new serotype. Different serotype-specific

¹²⁵I-peptides suggesting that simple post-translational modification of a basic, common protein does not account for generation of diverse serotypes. Monoclonal reagents have been developed to several serotype-specific proteins as well as to a "common" protein that probably represents axial filaments of these spirochetes (Barbour). The serotype-specific proteins are being sequenced (Barstad) to provide the basis for constructing mixed oligo nucleotide probes to study genetic mechanisms responsible for changes in serotype-specific proteins. It appears that these organisms should serve as valuable models for deciphering changes in exposed proteins by bacterial pathogens.

Surface components of Coxiella burnetii: Phase I (virulent) and phase II (avirulent) forms of C. burnetii were shown to differ with respect to both protein and LPS moieties. Phase I organisms, but not phase II, elicited a marked and persistent immunosuppression measured by lymphocyte proliferation in presence of phytomitogens. This immunosuppressive activity does not reside in chloroform-methanol extractable fractions of whole C. burnetii, and this chloroform-methanol extract holds promise as a less toxic but highly effective vaccine against Q fever. Monoclonal antibodies that differentiate phase I versus phase II C. burnetii were developed (Williams).

Lipopolysaccharide characterization by gel electrophoresis: Methods developed in LMSF for separation of bacterial LPS molecules by gel electrophoresis and LPS visualization by specific staining with silver proved highly reliable and efficient for defining structural differences among LPS moieties. Also developed was a method for proteinase K digestion of detergent solubilized whole bacteria. This allows rapid visualization of LPS structure by electrophoresis and silver staining without formal extraction of LPS. Correlation between biochemical compositions and gel profiles of the different Salmonella LPS chemotypes was impressive; this provided background data for examining the previously poorly characterized LPS's from various pathogenic bacteria. These methods provide new powerful ways for defining LPS structure (Hitchcock).

Virulence-associated characteristics of Bordetella spp: Many older studies documented the relationship between virulence of Bordetella pertussis and B. bronchiseptica with particular "phases" which were defined by colonial phenotypes of organisms grown on artificial medium; these phases have been dissected as to their biochemical and immunochemical characteristics during studies in LMSF over the past three years. From those results, it is clear that older phase-designations are inappropriate since they are inexact and each often represents a complex admixture of different characteristics. At present, B. pertussis and B. bronchiseptica have been carefully analyzed with regard to their outer membrane proteins, lipopolysaccharide compositions, flagellation, fimbriation, etc. The information generated by these analyses lays a solid groundwork for future selection of isogenic sets within a given species and strain of Bordetella such that individual characteristics of the organisms may be compared. This is a major contribution to understanding this group of organisms and will greatly enhance the abilities of future investigators to probe the pathogenic and virulence factors of Bordetella spp. which cause diseases in man and lower animals (Peppler).

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Laboratory of Microbial Structure and Function
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1982, to September 30, 1983

ADMINISTRATIVE REPORT

Personnel changes during the past year include departure of Mark Peppler (University of Alberta, Edmonton) and Jim Williams (USAMRIID, Fort Detrick, Frederick, MD) and arrival of three staff fellows: Paul Barstad, Ph.D., California Institute of Technology, Pasadena, CA; Frances Nano, Ph.D., University of Illinois, Urbana, IL; and Tim Howe, Ph.D., Oregon Health Sciences University, Portland, OR. Sohair Sabet, on sabbatical leave from Eastern Virginia Medical School, Norfolk, VA, joined LMSF as a Guest Worker. Summer students included Todd Damrow (University of Montana), Ramona Heiland (University of Utah Medical School), Albert Olszewski (Carroll College), Arturo Rivera (University of Utah), David Waag (University of Montana), and Timothy Wilson (University of North Dakota). Several scientists visited LMSF and presented seminars: Peter Ames, Northeastern University, Boston, MA; Patrick Bavoil, University of California San Francisco, CA; Charles Brinton, University of Pittsburgh, PA; Janne Cannon, University of North Carolina at Chapel Hill, NC; Robert Goldman, NIADDK, NIH, Bethesda, MD; Fred Hyde, University of Minnesota, Minneapolis, MN; Loretta Leive, NIADDK, NIH, Bethesda, MD; Greg McDonald, University of Virginia, Charlottesville, VA; Scott Minnich, Purdue University, Lafayette, IN; William Paranchych, University of Alberta, Edmonton; Gary Schoolnik, Stanford School of Medicine, Stanford, CA; and Trevor Trust, University of Victoria, Victoria, British Columbia.

Annual Report
Laboratory of Microbial Structure and Function
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1982, to September 30, 1983

HONORS AND AWARDS

Journal Editorial Boards:

J. Swanson - Journal of Bacteriology and Infection and Immunity

Manuscripts from Annual Review of Microbiology, Biochimica et Biophysica Acta, Canadian Journal of Microbiology, Infection and Immunity, Journal of Bacteriology, Journal of Immunology, Journal of Infectious Diseases, Science and Sexually Transmitted Diseases were also reviewed by members of LMSF staff.

Professional Posts:

J. Swanson - Ad hoc reviewer, Microbiology and Infectious Diseases Advisory Committee, Bethesda, MD

H. Caldwell - Convener, 5th International Society of STD Research, Seattle, WA

L. Mayer - Faculty Affiliate, Department of Microbiology, University of Montana, Missoula, MT
Presented 3 day Workshop on Recombinant DNA Techniques for Faculty Development Program, University of Montana, Missoula, MT

M. Peppler - Judge, Montana State Science Fair, Missoula, MT

J. Williams - Served as Consultant for an ad hoc review on Phase I Q Fever Vaccine (IND 610), USAMRIID, Fort Detrick, Frederick, MD
Co-chairman, Molecular Biology of Rickettsiae: RML Workshops in Molecular Microbiology, Hamilton, MT

Invited Lectures and Participation in Meetings and Symposia:

J. Swanson - Southern California Branch of American Society for Microbiology annual meeting, San Diego, CA
Agouron Institute, La Jolla, CA
University of Utah School of Medicine, Salt Lake City, UT
University of Montana, Missoula, MT
Workshop on Newer Aspects in the Development of a Vaccine for Gonorrhea, Bethesda, MD
EMBO Course on Molecular Genetics of Bacterial Pathogenicity, Umea, Sweden

A. Barbour - Fogarty Center, NIH, Workshop on Clone Concept of Infectious Diseases, Bethesda, MD
Bristol Lectureship, Combined Infectious Disease Sections of the Chicago Area Medical Schools, Chicago, IL
University of Montana, Missoula, MT
EMBO Course on Molecular Genetics of Bacterial Pathogenicity, Umea, Sweden

- H. Caldwell - International Symposium on Mechanisms of Bacterial Pathogenesis,
University of Tubingen, Tubingen, Germany
Canadian Society for Clinical Investigations, Calgary, Canada
University of Montana, Missoula, MT
Genetic Systems, Seattle, WA
University of Indiana, Indianapolis, IN
University of California San Francisco, San Francisco, CA
- T. Hackstadt - Molecular Biology of Rickettsiae: RML Workshops in Molecular
Microbiology, Hamilton, MT
- P. Hitchcock - Molecular Concepts of Lipid A, Walter Reed, Washington, DC
University of Utah School of Medicine, Salt Lake City, UT
University of Calgary School of Medicine, Calgary, Canada
Stanford University School of Medicine, Stanford, CA
- M. Peppler - University of Alberta, Edmonton, Alberta, Canada
University of Montana, Missoula, MT
Universal Foods Corporation, Milwaukee, WI
Montana Pharmaceutical Association, Helena, MT
- J. Williams - Victoria General Hospital, Halifax, Nova Scotia
Association of Military Surgeons of the U.S. 89th Annual Meeting,
Orlando, FL
University of Alabama, Birmingham, AL
Symposium series: Biological Implications of Pathogenicity,
University of Toronto, Toronto, Canada

Other Honors:

- J. Swanson - Reviewed research grants for National Science Foundation,
Washington, DC, and Medical Research Council of Canada,
Ottawa, Canada
- H. Caldwell - Reviewed research grants for British Columbia Health Care Research
Foundation, British Columbia, Canada
- J. Williams - Reviewed research grants for National Science Foundation,
Washington, DC

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00065-10 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antigens and Classification of Rickettsiae

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

R. L. Anacker, Res. Microbiologist, LMSF, NIAID

COOPERATING UNITS (if any)

Dr. K. E. Hechemy, New York State Dept. of Health, Albany, NY;

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are the determination of the nature and biological properties of rickettsial antigens and constituents and the development of procedures for classification of spotted fever group rickettsiae. SDS-PAGE analysis of four strains of rickettsiae representing three serotypes of the spotted fever group differed principally in the position of a major protein with an apparent molecular weight of about 17K. A sedimentable fraction of Sarkosyl-extracted rickettsiae contained predominantly a 17K and a 29K protein. Work is in progress to determine whether the 17K proteins are responsible for type specificity. A number of hybridomas producing antibodies which react with Rickettsia rickettsii in the indirect immunofluorescence test have been produced. One of these monoclonal antibodies protects mice against lethal challenge with R. rickettsii. The other reacts with a common epitope on multiple components of R. rickettsii; possibly this epitope is found on various classes of lipopolysaccharide molecules differing in the length of the polysaccharide side chains.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00182-05 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Genetical Mechanisms of Obligate Intracellular Parasitism

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

J. C. Williams, Scientist, LMSF, NIAID

COOPERATING UNITS (if any)

K.-I. Amano, Hirosaki University School of Medicine, Hirosaki, Aomori, Japan

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

0.9

PROFESSIONAL:

0.4

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective is to characterize some biochemical and genetic mechanisms of obligate intracellular parasitism of eukaryotic cells by Coxiella burnetii, the etiological agent of Q fever. This organism grows in the phagolysosomal milieu of eukaryotic cells. Thus, it resists the microbicidal mechanisms of this compartment. Previous studies have described a pathogenic mechanism of acid activation which leads to substrate transport and metabolism. More recently we have shown that in vitro protein synthesis occurs during axenic cultivation. However, multiplication of C. burnetii in complex media has not been achieved at this time. Some resistance properties of C. burnetii have been correlated with protease resistant proteins associated noncovalently and covalently to the peptidoglycan. Lysozyme hydrolysis of the polysaccharide portion of the peptidoglycan does not result in dissolution of the sacculus thereby conferring some resistance to microbicidal mechanisms. Moreover, stability of the peptidoglycan-protein complex may be enhanced by protein-protein and protein-peptidoglycan interactions between covalently- and non-covalently-bound proteins which are resistant to proteolysis. Phase specific monoclonal antibodies have been isolated which recognize phase I (virulent) or phase II (avirulent) cells. An objective is to use these monoclonals to monitor the frequency distribution of the phase variants. Perhaps we can understand some of the genetics involved in conferring resistance properties to C. burnetii. Future investigations into the molecular biology of C. burnetii will be carried out at the U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00183-05 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Bacterial Antigens in the Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

J. C. Williams, Scientist, LMSF, NIAID

COOPERATING UNITS (if any)

K.-I. Amano, Hirosaki University School of Medicine, Hirosaki, Aomori, Japan

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:

4.8

1.5

3.3

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective is to characterize the protective antigens and toxic components of Coxiella burnetii, the etiological agent of Q fever. Of primary importance is the biological and immunological characterization of subfractions of C. burnetii which confer immunological efficacy as measured by antibody and cellular mechanisms. Appropriate subfractions are being analyzed as candidates for subunit vaccines. Virulent and avirulent C. burnetii are represented by phase I and II cells, respectively. Surface-exposed protein species revealed three distinct differences between phase I and II. As a consequence of phase variation protein, glycoprotein, surface protein and lipopolysaccharide species show marked differences between the phase variants. Although differences in immunogenic proteins were detected between the phase variants, extensive homology existed. Thus, the variable pathogenic potential of the phase variants may be related to key immunogenic proteins as well as phase I lipopolysaccharide. Phase I whole cell vaccine, but not phase II, induced marked and persistent suppression of the proliferative response of mouse spleen cells to phytomitogens. The effect was time and dose dependent, and was due to a true hyporesponsive state of the host cells. Spleen cells from mice injected with this vaccine were down-regulated, in as much as cellular, DNA, RNA, and protein synthesis were depressed by homologous antigen. The suppressive activity of the vaccine was removed by chloroform-methanol extraction of phase I whole cells. The chloroform-methanol residue induces immunity, however, the pathogenic potential is reconstituted by mixing chloroform-methanol extract with the chloroform-methanol residue. The chloroform-methanol residue is currently being developed as a second generation vaccine for human use. Future investigations into the development of an improved Q fever vaccine for human use will be carried out at the U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00193-04 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gonococcal Surface Components: Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

J. Swanson, Chief, LMSF, NIAID

COOPERATING UNITS (if any)

K. Joiner and M. Frank, LCI, NIAID, Bethesda MD

M. Blake and E. Gotschlich, Rockefeller University, New York, NY

M. Koomey and G. Schoolnik, Stanford University, Stanford, CA

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: PROFESSIONAL:

1.1

0.6

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is definition of structural differences and similarities of selected surface-exposed constituents of gonococci from various strains and intrastrain variants. During the current year, results have yielded a modified model for disposition of outer membrane protein I; protein I constituents from organisms causing disseminated gonococcal infections were found to expose only one end of their subunits to the external environment. This contrasts with the protein I subunits of organisms causing local gonorrheal infections inasmuch as these forms of protein I seem to have a "hairpin" orientation in the outer membrane with a mid-portion of these molecules being exposed on the organisms' surfaces. The major effort on this project during the past year has been definition of the structural variations in pilus subunits that can be delineated among variants of individual gonococcal strains. It was found that at least a dozen different subunit forms (by size) of pili occur among these intrastrain variants. These findings predict that formulation of a pilus-based vaccine for gonorrhea will have to include many more serotypes of pili than was previously appreciated. Genetic mechanisms responsible for both pilus expression and for variation in pilus subunit form are being investigated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00194-04 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of *Neisseria gonorrhoeae*

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

L. W. Mayer, Expert (Microbiology), LMSF, NIAID

COOPERATING UNITS (if any)

Dr. Mel Simon, University of California at San Diego, San Diego, CA

Dr. Milan Blake, Rockefeller University, New York, NY

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Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

1.9

PROFESSIONAL:

0.9

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The emphasis of this project is to use molecular genetics, including recombinant DNA techniques, to understand the pathogenesis of gonorrhea and some other infectious diseases. We have developed and are characterizing monoclonal antibodies to several gonococcal outer membrane proteins. Preliminary data suggest that we have antibodies which are strain specific and some which react with the protein of several strains. These antibodies will be used to screen our clone banks and identify the genes of interest. We have also synthesized a mixed oligonucleotide "probe" which should detect the structural gene for the major outer membrane protein (PI) of the gonococcus. This mixture of oligonucleotides matches the sequences which could code for one of the amino acid sequence portions which have been determined for his molecule. Since several of the systems studied at RML may consist of multi-gene families, we have developed a novel cloning vector which allows selection of genes by homology. This system is similar to that utilizing lambdoid phage developed by Brian Seed but has two significant advantages.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00196-04 LMSF

PERIOD COVERED

October 1, 1982, to August 12, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bordetella pertussis: Outer Membrane Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

M. S. Peppler, Expert (Microbiology), LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	0.9	0.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project's purpose is to define the biochemical composition of the major surface constituents of Bordetella pertussis and their relationship to the pathogenicity and epidemiology of whooping cough. Techniques have been developed which allow for the controlled selection, from a single strain, of the three major, stable phenotypes of B. pertussis. Isogenic sets of cloned phenotype variants are compared for differences in SDS-PAGE profiles after Coomassie brilliant blue staining for protein, extrinsic labeling by ¹²⁵Iodine and/or silver staining for lipopolysaccharide. The three phenotypes possess characteristic SDS-PAGE profiles, colonial morphologies on BGA, growth characteristics on nutrient agar and antibiotic and oleic acid sensitivity profiles. Lipopolysaccharide variants have been characterized and their role in phenotype dynamics was studied. Two lipopolysaccharide types have been defined, isolated, and characterized chemically and immunologically. Analysis of specific mutants and their products can better define the genetic basis of virulence in B. pertussis for furthering understanding of pathological and immunological mechanisms of whooping cough.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00216-03 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunochemistry of Chlamydial Surface Antigens

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

H. D. Caldwell, Expert (Microbiology), LMSF, NIAID

COOPERATING UNITS (if any)

Dr. J. Schachter, University of California Medical Center, San Francisco, CA

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Laboratory of Microbial Structure and Function, Hamilton, MT 59840

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.7	0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous work on this project has shown that the major outer membrane protein (MOMP) of Chlamydia trachomatis organisms is the serotyping antigen of this important group of human pathogens. It was also shown that polyclonal IgG antibody specific for the MOMP neutralized chlamydial infectivity in vitro and that this neutralization was primarily serotype specific. Currently this project is focused on the identification and molecular characterization of these potentially important surface antigens of C. trachomatis organisms. The approach has been to raise monoclonal antibodies against serotype and serogroup epitopes present on the MOMP. These antibodies were radioiodinated and used as probes to map the native antigenic topography of MOMP on viable chlamydiae. The data show that only monoclonal antibody directed against the serotype-specific epitope of the MOMP is capable of binding to the surface of viable organisms. These same antibodies were also found to reduce in vitro infectivity of the parasite. In contrast, radioiodinated monoclonal antibodies against a species-specific epitope of the MOMP or a genus-specific epitope located on chlamydial lipopolysaccharide did not bind viable chlamydiae or reduce infectivity. These data suggest that the surface-exposed antigenic domains of the MOMP that confer serotype specificity are promising candidate antigens for a C. trachomatis subunit or peptide vaccine. A 9K dalton cyanogen bromide fragment of MOMP that binds to the MOMP serotype-specific monoclonal antibody has been identified by immunoblotting analysis (see project #Z01 AI 00233-02 LMSF). This peptide fragment is being isolated by immunosorption using solid phase bound type-specific monoclonal IgG antibody. Once isolated, the amino acid sequence of the peptide or its succinylated-trypsin digested fragments will be determined. It is then proposed to synthesize the immunological active peptide and to initiate appropriate animal model studies to evaluate its vaccine potential.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00230-02 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Virulence-Associated Factors of Rickettsia rickettsii

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

R. L. Anacker, Research Microbiologist, LMSF, NIAID

COOPERATING UNITS (if any)

None

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NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
1.5 0.5 1.0

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Five strains of Rickettsia rickettsii from Montana and North Carolina were examined in an effort to identify rickettsial constituents associated with virulence for guinea pigs. The HLP strain of least virulence was differentiated from the other strains by SDS-PAGE followed by staining with Coomassie brilliant blue or with silver. The Montana patient strains of highest virulence could not be distinguished from the North Carolina patient strains of lesser virulence by these procedures. All of the strains apparently had the same heat-modifiable proteins. Analysis of proteinase K-digested rickettsial lysates by SDS-PAGE suggested that the strains had a complex mixture of polysaccharides. These putative polysaccharides probably were not responsible for the differences in virulence of the strains, since the patterns for all of the strains were identical. At least five antigens were demonstrated in all strains by radioimmune precipitation tests. In immunoblotting tests, the HLP strain differed from the other strains in the presence of a 52K antigen; the other strains had a 51K antigen instead.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00231-02 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology of Relapsing Fever Borrelia spp.

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

A. G. Barbour, Senior Staff Fellow, LMSF, NIAID

COOPERATING UNITS (if any)

Prof. M. Simon, California Institute of Technology, Pasadena, CA

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Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK. (Use standard unreduced type. Do not exceed the space provided.)

The mechanism of the antigenic variation shown by the relapsing fever borreliae is under study. Having identified serotype-specific, molecular weight-variable proteins at the surface of Borrelia hermsii, we analyzed the structure of these serotype-specific proteins (SSP) by peptide mapping procedures and immunochemical techniques. The SSPs differed considerably in their peptide maps and in their reactions with monoclonal antibodies and polyclonal antisera. This apparent paucity of amino acid sequence homology suggests possible models for the mechanism underlying SSP variability. In our continuing investigations of the genetic mechanism of borrelial antigenic variation, we characterized the genome of B. hermsii and identified extrachromosomal elements in the HS1 strain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00232-02 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Biology of Ixodes spp. Tick Spirochetes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

A. G. Barbour, Senior Staff Fellow, LMSF, NIAID

COOPERATING UNITS (if any)

Dr. A. C. Steere, Yale University School of Medicine, New Haven, CT

Dr. J. Benach, State University of New York at Stony Brook, and New York
State Department of Health, Stony Brook, NY

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SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
1.1 0.5 0.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Ixodes spp. tick-associated spirochetes were isolated during the preceding year from the blood, skin, and cerebrospinal fluid of Lyme disease patients. This finding together with demonstration of antibody responses by Lyme disease patients to several components of the spirochetes indicates that the tick-associated spirochetes are the etiological agents of Lyme disease. With biochemical and immunochemical techniques, we are identifying the major antigens of these spirochetes and studying the pathogenesis of Lyme disease.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00233-02 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Analysis of the Major Outer Membrane Protein of Chlamydia trachomatis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

H. D. Caldwell, Expert (Microbiology), LMSF, NIAID

COOPERATING UNITS (if any)

None

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Laboratory of Microbial Structure and Function, Hamilton, MT 59840

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INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this work is to identify, isolate, and chemically define the peptide fragment of the chlamydial major outer membrane protein (MOMP) which contains the antigenic determinant that confers Chlamydia trachomati serotyping specificity. These antigenic determinants of the MOMP are exposed on the surface of viable chlamydiae and elicit neutralizing antibody in vitro; therefore they are attractive candidates for chlamydial vaccine development (see project Z01 AI 00216-03 LMSF). Purified chlamydial MOMP was digested with cyanogen bromide (CNBr) or succinylated and trypsin digested. The antigenic properties of the peptide fragments were analyzed by immunoblotting with monoclonal antibody directed against the serotype-specific epitope of the intact protein. A single 9K dalton CNBr fragment was identified that reacted with the serotype specific monoclonal antibody. This peptide is currently being isolated by immunoadsorption using solid phase bound antibody. Amino acid sequencing of the 9K fragment and the parent MOMP have been performed. This information will be used to generate a synthetic oligonucleotide probe and to construct synthetic peptides. This should allow molecular cloning of the peptide gene and its synthetic biosynthesis which will provide the needed quantities of the peptide to begin experimental in vivo animal model vaccine studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00234-02 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology of Intracellular Parasitism

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

T. Hackstadt, Staff Fellow, LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is devoted to the basic biology of intracellular parasite-host cell interaction. Representative obligately intracellular prokaryotes occupying different intracellular compartments within the eucaryotic cell are being examined to allow comparison of the varied mechanisms employed by these microorganisms to allow survival and growth within eucaryotic cells. In the past year, the bioenergetic properties of Coxiella burnetii, the etiological agent of Q fever, have been described. These studies demonstrate a physiological basis for the extreme resistance of C. burnetii to environmental conditions while providing for an activation of metabolism upon ingestion into the acidic and normally bactericidal phagolysosome. Currently, initial events involved in the interaction of parasites with the host are under investigation. The following observations have been made: (i) the major outer membrane protein (MOMP) of chlamydia is extensively cross-linked via disulfide bands, (ii) the extent of this cross-linking varies between the infectious elementary body and the differentiated reticulate body, and (iii) cleavage of surface-exposed portions of this protein by a variety of proteases did not reduce infectivity or rates of association with tissue culture cells. From these studies, it was concluded that structural stability of chlamydia, which had peptidoglycan, was conferred by cross-linking of MOMP, but that surface-exposed protease sensitive portions of this protein are not required for attachment, entry, blockage of phagosome-lysosome fusion or differentiation to the replicating reticulate body.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00235-02 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of Gonococcal Lipopolysaccharide

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

P. Hitchcock, Staff Fellow, LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.35	0.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is comprised of studies relating to the structure and function of gonococcal lipopolysaccharide (LPS), with particular emphasis on the role of LPS in the pathogenesis of gonorrhea. Studies have focused on the molecular heterogeneity of LPS as analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the interaction of proteins and lipopolysaccharide, the biochemical analysis of LPS and characterization of the antigenicity and immunogenicity of gonococcal LPS with rabbit antisera elicited against viable gonococci, against gonococcal outer membrane protein I, against free lipid A, and *Salmonella* Re chemotype and with normal human sera using immunoelectroblotting.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00236-02 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Biology of Contagious Equine Metritis Organism

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

P. Hitchcock, Staff Fellow, LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

0.6

PROFESSIONAL:

0.35

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project concerns characterization of the surface components of a recently defined veterinary pathogen: the causative agent of contagious equine metritis (CEM). CEM is a sexually transmitted venereal disease of horses; the etiological agent is an unclassified gram-negative diplococcus which shares several morphological and biochemical characteristics with Neisseria species. In addition, the disease profile of CEM bears some resemblance to gonorrhea in humans. Continued studies focus on (1) evaluation of colony morphology, (2) evaluation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of outer membrane constituents: lipopolysaccharide, capsular polysaccharide and proteins, and (3) evaluation of antigenic composition of surface constituents using hyperimmune rabbit sera and equine immune sera from experimentally infected animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00238-02 LMSF

PERIOD COVERED

October 1, 1982, to August 12, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bordetella pertussis and Bordetella bronchiseptica: Outer Membrane Structure

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

M. S. Peppler, Expert (Microbiology), LMSF, NIAID

COOPERATING UNITS (if any)

Dr. David Bemis, University of Tennessee, Knoxville, TN

Dr. Gail Cassell, University of Alabama, Birmingham, AL

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Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was established to apply the knowledge and techniques acquired while assessing virulence-associated components in B. pertussis to B. bronchiseptica. Isogenic sets of colonial phenotype variants are selected, cloned, and compared on three media for phenotype stability. Particular attention is paid to flagella expression, assessed by light, and scanning electron microscopy, and to ¹²⁵I-surface labeling and lipopolysaccharide profiles on SDS-PAGE. Antisera are raised in mice to determine "phenotype specific" components by double diffusion, radio immune precipitate, and transfer-blotting techniques. Growth of different phenotypes in cell culture correlates pathogenicity with cytotoxicity. Both the in vitro cell culture system and in vivo infection of rats are used to assess the relative virulence of different strains and their phenotypes. By conducting studies with isogenic sets of phenotypes from different strains, the mechanisms and attributes of B. bronchiseptica infections can be studied in a more genetically controlled manner.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00239-02 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gonococcal Surface Proteins' Immunochemical Characteristics

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

J. Swanson, Chief, LMSF, NIAID

COOPERATING UNITS (if any)

Keith Joiner, LCI, NIAID, Bethesda, MD

Milton Tam, Genetic Systems, Seattle, WA

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Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
1.0 0.5 0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to define the immunochemical characteristics of selected components that are exposed on the exteriors of gonococci. This information is intended to provide background data on which design of vaccines to prevent gonorrhea can be based. During the past year, the project has concentrated on study of two groups of outer membrane proteins--protein III and protein II. The protein III constituents were found to be constant in presence and immunochemical reactivity among all strains and substrain variants of gonococci. The protein III moieties are surface-exposed and are potential targets for bactericidal actions of antibodies. Proteins of identical immunochemical reactivities as gonococcal protein III but different (from gonococci) subunit sizes were found on several other neisseriae including both meningococci and commensal species. Outer membrane protein II moieties were found to be highly variable both in occurrence and immunochemical characteristics on variants of individual strain and among diverse strains. All protein II forms exhibit common antigenicities, but these common regions appear buried on the organisms' outer membranes; exposed portions of protein II appear to be unique, in general, though some examples of cross-reactivities were found both within a given strain and among different strains. More than one protein II can coexist on individual gonococci.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00362-01 LMSF

PERIOD COVERED

November 28, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Primary Structural Analysis of Bacterial Membrane Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

P. A. Barstad, Senior Staff Fellow, LMSF, NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.9	OTHER: 0
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CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to obtain sufficient partial sequence analysis from bacterial surface membrane proteins to allow construction of synthetic oligonucleotide probes with which the genes encoding the proteins can be isolated. The genes will then be sequenced by standard methods and may also be used to generate pure material from Escherichia coli for vaccine development. Milligram amounts of surface proteins from Borrelia hermsii and Chlamydia trachomatis have been purified by preparative SDS-gel electrophoresis and examined by automated sequence analysis. These preliminary analyses indicate that appropriate sequences for probe construction are located in the N-terminal region of the Borrelia pII protein and the Chlamydia L-2 major outer membrane protein (MOMP). In contrast, none of the PI proteins sequenced well in the Beckman system. Techniques directed at selective purification of probe-compatible peptides by HPLC from the PIs are currently being developed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00363-01 LMSF

PERIOD COVERED

June 1, 1983, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Obligate Intracellular Bacteria as the Etiological Agent of AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

H. D. Caldwell, Expert (Microbiology), LMSF, NIAID

COOPERATING UNITS (if any)

G. Byrne, University of Wisconsin Medical School, Madison, WI

LAB/BRANCH

Laboratory of Microbial Structure and Function, Hamilton MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this work is the characterization of an obligate intracellular procaryote that has recently been isolated from monocytes of patients with acquired immune deficiency syndrome (AIDS). This agent is of interest because it has been shown to be very similar to Ehrlichia canis, the causative agent of canine ehrlichiosis, a disease in which the agent grows in monocytes of dogs resulting in an immuno-compromised condition that is most often fatal. E. canis is currently classified in the Rickettsiales, however ultrastructural studies have shown that the agent grows within a cytoplasmic endosome, a property shared with Chlamydia and Coxiella, not the Rickettsias. These observations suggest that the agent may be closely related to Chlamydia. The purpose of this study is to determine if the AIDS procaryote is related to the chlamydial group of organisms. The approach will be the following: (i) Propagate the agent from infected human monocytes into McCoy cells using standard methodology for the isolation and growth of Chlamydia. (ii) Perform ultrastructural studies of both infected cells and organisms isolated from infected cultures. These studies will describe the intracellular compartment of growth, agent morphology and its growth cycle properties. (iii) Immunological analysis with characterized monoclonal antibodies reactive against Chlamydia and Coxiella. (iv) DNA-DNA hybridizations with both chlamydial and Coxiella burnetii DNA and G+C ratios. (v) Metabolic and physiological studies to determine if the agent grows within the phagosome or phagolysosome of the cell to distinguish between Chlamydia and Coxiella. (vi) Introduction of the agent into various animal species with the goal of developing a model of immuno-compromised infections such as AIDS and ehrlichiosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00364-01 LMSF

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of Lipopolysaccharide by SDS-Polyacrylamide Gel Electrophoresis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

P. Hitchcock, Staff Fellow, LMSF, NIAID

COOPERATING UNITS (if any)

None

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NIAID, NIH, Bethesda, MD 20205

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1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is comprised of studies relating to the characterization of bacterial lipopolysaccharides (LPSs) by sodium dodecyl sulfate-polyacrylamide gel system. LPSs exhibit marked heterogeneity in silver-stained polyacrylamide gels. Using biochemically defined LPS chemotypes of Salmonella sp., we have shown that the morphological heterogeneity of LPS gel profiles correlates positively with biochemical variation in LPS composition. Development of several techniques including a silver stain which preferentially stains LPS in bacterial whole cell lysates, proteinase K digestion of whole cell lysates, and two-dimensional electrophoresis of LPS in polyacrylamide gels has enabled us to utilize SDS-PAGE as an effective tool for fingerprinting bacterial LPSs.

Laboratory of Persistent Viral Diseases
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Hamilton, Montana
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Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1982 to September 30, 1983

RESEARCH HIGHLIGHTS

Mechanisms of action of Rfv-1 gene. The Rfv-1 gene in the H-2D region has been shown to influence the kinetics of development of the Friend virus-specific T lymphocyte response. The high recovery genotype H-2D^{b/b} mice respond 6-10 days faster than the low recovery H-2D^{d/b} mice. The kinetic difference appears to account for the differences in recovery incidence (Chesbro).

Lack of MCF virus expression in certain mouse leukemias. Use of a sensitive MCF-specific monoclonal antibody with conventional virus isolation methods on cells from leukemic organs and in vitro lines derived from leukemic organs indicates that MCF virus expression may not be required for leukemogenesis in certain mouse strains or hemopoietic lineages (Chesbro, Portis).

Bone marrow suppression in graft versus host disease. A single dominant non-H-2 gene has been found in C57BL/6 mice which promotes bone marrow suppression during the acute reaction which occurs in F₁ recipients given $\geq 60 \times 10^6$ C57BL/6 spleen cells (Portis).

Monoclonal antibodies identify retroviral envelope antigens on differentiating cells. Monoclonal antibodies with specificity for different xenotropic murine leukemia viruses were derived from mice undergoing graft versus host reactions. Some of these antibodies also recognized viral antigens on uninfected hemopoietic cells at different stages of differentiation. This finding suggested that expression of retroviral gene sequences may be involved in the normal differentiation process (Portis).

Two mouse genes influence spontaneous recovery from rabies virus infection. Testing of first and second backcross progeny has confirmed that two mouse genes are involved in recovery from intraperitoneal inoculation of rabies virus. Other genetic crosses have shown that resistance genes of two different mouse strains, SJL and CBA, are allelic (Lodmell).

Frequency of scrapie virus infected cells was determined in mouse spleen cell populations. 0.1-0.01% of spleen cells from scrapie infected mice were found to be virus-infected by direct titration of washed cells (Race, Chesbro).

Endogenous sequences present in MCF recombinant viruses. Endogenous gene sequences involved in recombination with different ecotropic retroviruses (Friend, Moloney and AKV) could be distinguished by oligonucleotide finger-printing. Furthermore, the inclusion of ecotropic rather than endogenous p15(E) sequences in recombinants appeared to depend on the type of ecotropic virus used. Many recombinant viruses derived from Friend ecotropic virus have most of the gag and pol regions contributed by the endogenous parental sequences (Evans, Cloyd).

Regulation of FP synthesis. Hamster FP, a homolog of human CRP, was found to be synthesized in the liver under control of sex hormones. In acute inflammatory reactions, a sex limited divergent synthetic response was observed (Coe).

Expression of ADV antigens in E. coli. Molecular clones in plasmids pUC8 from two strains of ADV expressed viral antigens in *E. coli*. These antigens reacted with sera containing anti-ADV antibodies by Western blotting and immune precipitation. In addition, inoculation of animals with lysates of recombinant clones induced antibody to viral proteins (Bloom, Aasted, Mayer).

Localization of replicate forms of ADV DNA in lymphoid cells. Using molecularly cloned ADV DNA as a probe, replicative forms of viral DNA were localized in gut, liver, spleen and lymph node cells of infected mink. This is the first demonstration of the site of ADV replication in organs of infected mink (Bloom).

Mink with AD have high affinity antiviral antibody of restricted heterogeneity. Determinations on IgG from sera of infected mink using an RIA showed that mink develop high affinity antibody directed against virion antigens. Additional studies suggested that some mink developed antiviral antibodies with highly restricted heterogeneity (Aasted, Bloom).

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Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1982 to September 30, 1983

ADMINISTRATIVE REPORT

New personnel added to the LPVD staff during the past year included: Dr. Marc Sitbon, Visiting Fellow from Paris, France, working on mechanisms of resistance to murine leukemia with Dr. Chesebro; Dr. Howard Etlinger, a Senior Staff Fellow, working on basic immune mechanisms and their role in resistance to viral diseases; Dr. Steven Palmieri, a Staff Fellow, working on transformation of cells by avian erythroblastosis virus; Dr. Anders Cohn, a Guest Worker from Copenhagen, Denmark, working on Aleutian disease virus with Dr. Bloom.

Two scientists spent the summer working at LPVD: Dr. David Hankins, NCI, Bethesda, MD and Dr. Ashley Haase, University of California at San Francisco.

Summer students in the LPVD were: Mel Margaris, Greg Tierney and David Olsen, Carroll College, Helena, MT; Joseph Lucero, Idaho State University, Pocatello, ID; Ronald Smith, Oregon State University, Corvallis, OR.

Annual Report
Laboratory of Persistent Viral Diseases
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1982 to September 30, 1983

HONORS AND AWARDS

Editorial Boards of Journals:

Dr. B. Chesebro

Associate Editor - Journal of Immunology

Dr. D. L. Lodmell

Associate Editor - Journal of Immunology

Professional Posts:

Dr. B. Chesebro - Adjunct Professor - Department of Microbiology,
Montana State University, Bozeman, MT

Dr. M. E. Bloom - Faculty Affiliate, Department of Microbiology,
University of Montana, Missoula, MT

Dr. J. E. Coe - Faculty Affiliate, Department of Microbiology,
University of Montana, Missoula, MT

Dr. D. L. Lodmell - Faculty Affiliate, Department of Microbiology,
University of Montana, Missoula, MT

Invited Lectures at Meetings or Symposia:

Dr. M. E. Bloom - Co-organizer of the Colloquium on Mink Virus Diseases, RML,
Hamilton, MT

Dr. J. E. Coe - Rocky Mountain Immunology Association, Taos, NM

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00074-11 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetically Controlled Mechanisms of Recovery from Friend Virus-Induced Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

B. Chesebro

Chief

LPVD NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda MD 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
2.5 1.5 1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recovery from Friend virus (FV)-induced erythroleukemia in mice is influenced by two H-2-linked genes, Rfv-1 and Rfv-2, and one non-H-2 associated gene, Rfv-3. The Rfv-1 and Rfv-2 genes have now been found to influence the virus-specific T lymphocyte response. The Rfv-2 gene has been mapped to the I region of the H-2 complex and appears to function as a dominant immune response gene. The Rfv-1 gene, located in the D region of H-2, influences the kinetics of development of the FV-specific T lymphocyte proliferative response. Previous assays for FV-specific T lymphocyte function such as cell-mediated cytotoxicity were always negative in mice which had not recovered. However, the use of an *in vitro* T lymphocyte proliferation test was able to detect a positive response in some mouse strains even in the presence of leukemic splenomegaly. Using this test, mice with the high recovery genotype (H-2D^{d/b}) had a positive T lymphocyte response 6-10 days earlier than mice with the low recovery genotype (H-2D^{r/b}). This result was also supported by the fact that adoptive transfer of immune T lymphocytes induced recovery from leukemia in H-2D^{d/b} mice only if transferred early after virus inoculation. Thus, the delay in the development of the FV-specific T lymphocyte response of H-2D^{d/b} mice apparently allowed the virus-induced leukemia to progress to the point where the leukemia could no longer be eliminated by the intrinsic T lymphocyte response or by passively transferred immune T lymphocytes. The Rfv-3 gene influences production of anti-FV antibody, and has been found to be necessary but not sufficient for recovery from leukemia. However, in the absence of the appropriate Rfv-1 (H-2D^{b/b}) genotype, Rfv-3^{r/s} mice still make antibody which leads to elimination of FV viremia and marked reduction in virus release by leukemic spleen cells. Recent results using passive transfer of monoclonal antibodies showed that the Rfv-3 gene effects could be mimicked only by anti-gp70 antibodies of the IgG_{2a} subclass. These antibodies appeared to mediate selection and overgrowth of spontaneously arising virus nonproducer cells in the leukemic spleen cell populations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00260-02 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Role of Endogenous and Recombinant Retroviruses in Leukemia and Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

B. Chesebro

Chief

LPVD NIAID

COOPERATING UNITS (if any)

None

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NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mouse monoclonal antibodies reactive with gag or env proteins of Friend murine leukemia virus (F-MuLV) or recombinant MCF viruses related to F-MuLV were derived and characterized for viral protein specificity and reactivity with a large panel of MuLV's. Analysis of 10 strains of F-MuLV distinguished seven different antigenic phenotypes, all of which retained reactivity for three anti-gp70 antibodies uniquely specific for Friend and Rauscher MuLV's. Four antibodies with a unique reactivity for recombinant MCF viruses were also found. Most known MCF viruses react with at least one or two of these MCF-specific antibodies. F-MuLV inoculation of newborn mice was previously shown to induce leukemias in certain mouse strains. We have found that the predominant cell type in leukemia organs differed markedly in various mouse strains. Using a monoclonal anti-MCF virus antibody test as well as conventional MCF virus isolation, we observed a good correlation between appearance of MCF virus and presence of erythroid, myeloid or lymphoid leukemia in IRW mice. In contrast, in C57BL/10 mice only lymphoid and myeloid leukemias were observed and infectious MCF viruses were never isolated. In (C57BL/10 X IRW)F₁ mice, erythroid, myeloid, lymphoid and mixed leukemias were seen, but MCF viruses were isolated from only 25% of the mice. These results indicated that generation or expression of MCF viruses may not be necessary for leukemogenesis in some mouse strains or hematopoietic lineages.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00072-12 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Host and Viral Factors in Resistance to Rabies Virus Infections in Mice

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

D. L. Lodmell

Scientist Director

LPVD NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

2.2

PROFESSIONAL:

0.8

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|---|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major objective of this project is to determine host and viral factors which influence murine resistance to rabies virus. It has been shown that resistance to intraperitoneally (i.p.)-inoculated street rabies virus (SRV) was under genetic influence. Testing of second backcross progeny has confirmed that two genes controlled susceptibility if the first backcross parent had been selected for resistance to SRV and both parents were of similar H-2^S haplotype. If the parents were of different H-2 haplotypes, or the first backcross parent was randomly selected, different combinations of 1-4 segregating genes appeared to be involved. This secondary influence of H-2 was not obvious in earlier studies with inbred strains and F₁ hybrids. Additional genetic studies have indicated that SRV resistance genes of SJL/J and CBA/J mice were allelic (identical). Pathogenesis studies have shown that 5 days after i.p. inoculation SRV entered the spinal cord of resistant and susceptible mice in the thoracic area, probably along splanchnic nerves of the sympathetic nervous system. Following entry into the thoracic area, SRV ascended to the cervical area of the cord and then the brain. Minimal concentrations of virus were detected in the lumbar area. There was minimal replication of SRV in spinal cord and brain of resistant SJL and CBA mice. In contrast, virus concentrations were 99% greater in CNS tissues of susceptible mice as compared to resistant mice which developed clinical disease but survived. Serum neutralizing antibody appeared earlier and the titer was 100- to 1000-fold greater in resistant SJL and CBA as compared to susceptible mice. DBA/2 mice, however, which survived after onset of clinical disease, had neutralizing antibody titers similar to susceptible strains. Studies of immunoglobulin isotypes have indicated there was a higher concentration of Y_{2a} in sera of uninfected resistant SJL as compared to susceptible A/WySn mice. Immune function defects are being introduced into resistant mice to help elucidate the reasons for susceptibility differences to SRV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00261-02 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunological Aspects of Neurovirology

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

D. L. Lodmell

Scientist Director

LPVD NIAID

COOPERATING UNITS (if any)

None

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SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major objective of these studies is to delineate the importance of immune responses within the central nervous system (CNS) of mouse strains which vary in their resistance to neurotropic rabies virus. It has been determined that rabies virus neutralizing antibody was not present in the cerebrospinal fluid (CSF) of resistant or susceptible strains of mice 3, 5, 7 or 10 days following intraperitoneal inoculation of street rabies virus (SRV). Antibody was present, however, in resistant strains on the 15th day and thereafter, but seldom was detected in the CSF of susceptible A.SW, A/WySn or athymic (nude) mice. It is not known whether the paucity of neutralizing antibody in the CSF of these mice correlated with their susceptibility, or whether the delayed appearance of antibody in the CSF of resistant mice (>10 days) was irrelevant as a mechanism of resistance to infection. Furthermore in resistant mice, there was no correlation between CSF antibody titer and the clinical course of disease; mice which developed clinical signs of illness but survived (DBA/2, BALB/c) had titers similar to mice which did not develop clinical illness (SJL, CBA/J). In addition, antibody never was detected in the CSF of mice which had serum neutralizing antibody titers of <1:320. The future course of this project will focus on understanding the protective, if any, role of neutralizing antibody in the CSF and whether it is synthesized locally in the CNS. In addition, the importance of interferon and T-lymphocytes in the CSF will be assessed in mice strains which vary in their susceptibility to SRV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00073-18 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Immunity and Immunopathology Related to Cellular/Humoral Immunity

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

J. E. Coe

Medical Officer

LPVD NIAID

COOPERATING UNITS (if any) Dr. Kamal Ishak, AFIP, Washington, DC; Dr. G. Genovesi and Dr. C. J. Peters, U.S. Army, Medical Research Unit of Infectious Diseases, Fort Detrick, Frederick, MD; Dr. R. F. Schell, Div. Infect. Dis., Hahnemann Medical College, Philadelphia, PA; Dr. S. Reed, Cornell Medical Center, New York, NY

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1.0

0.5

OTHER:

0.5.

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Syrian hamster is an especially suitable host for several infectious diseases. This laboratory is studying the inflammatory and immune response in various hamster infectious disease models which are ongoing in other investigators' laboratories. We are interested in any involvement (helpful or injurious) of acute phase reactants (female protein) and also wish to determine the isotype of antibody and correlate these factors with the resultant immunopathology. A persistent viral infection (LCM) is being studied in inbred hamster strains, which are either susceptible (MCH) or resistant (CB). In a persistent treponema model, hamsters have been found to be a suitable host for syphilis and yaws and protective immune immunoglobulins have been isolated and identified. One parasitic model also is being studied, as leishmania produces a chronic disease in hamsters similar to human infection; we have found evidence for glomerular immune complexes and also marked amyloid deposition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00262-02 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Pentraxins in Acute and Chronic Pathology

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

J. E. Coe Medical Officer LPVD NIAID

COOPERATING UNITS (if any) Dr. H. Gewurtz, Rush Med. College, Chicago, IL; Dr. D. Briles, Dept. Cell. Immunobiol., Univ. Alabama, Birmingham, AL; Dr. J. Sogn, NIAID, Bethesda, MD; Dr. S. Margossian, Bronx, NY; Dr. K. McAdam, Tufts Univ., Boston, MA; Dr. S. S. Mookerjea, Mem. Univ. of Newfoundland, St. Johns, Newfoundland

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1.0	0.5	0.5

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A homolog of human C-reactive protein (CRP) and amyloid Pcomponent (AP) is present in the Syrian hamster as a sex limited serum protein called female protein (FP). FP functionally is similar to both human proteins as it has a Ca⁺⁺ dependent phosphorylcholine binding site like CRP and is a component of amyloid similar to AP. This protein is a glycoprotein and its synthesis in the liver is controlled by sex hormones and acute phase mediators. Inflammation results in decreased synthesis of FP in females, whereas increased synthesis of FP is found in males. This divergent acute phase response is controlled by levels of testosterone and estrogen in the injured hamster. FP is a noncovalently assembled pentamer of 30K dalton subunits. The monomer subunits are hydrophilic and can reassemble to form a pentamer indistinguishable from the parent molecule.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00085-06 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis of Aleutian Disease Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

M. E. Bloom

Medical Officer

LPVD NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.8	0.4

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is the study of Aleutian disease of mink (AD), a persistent infection by the Aleutian disease virus (ADV), a nondefective parvovirus. In the past year, we have utilized reagents and probes to begin detailed study of AD, both *in vivo* and *in vitro*. Using ADV-G infected Crandell feline kidney (CRFK) cells as an *in vitro* model, we have quantitated infectious virus, viral antigens (both by fluorescence and radioimmunoassay) and viral DNA (by dot-blot hybridization). The data generated by these experiments have allowed us to conclude that an infected CRFK cell expressing ADV antigens contains $\sim 10^5$ genome copies of viral DNA. Relating this information to data on viral DNA content of tissues of infected mink, we have determined that at the height of viral replication (10 days after infection with Utah I ADV), approximately $\sim 0.02\text{-}0.1\%$ of the cells in mesenteric lymph node, spleen and liver contain virus. In addition, analysis of Southern blot hybridization has localized ADV DNA with characteristics of replicative forms in these tissues, implying they are true sites of replication. Additional studies suggest that the virus is located in cells of the lymphocyte series and not (exclusively) present in phagocytic cells. Current studies are focussing on cells isolated from mesenteric lymph node, and we are presently analyzing lymphocyte populations highly enriched for "T" or "B" lymphocytes. Additional work has suggested that the small MW proteins found in ADV isolated from mink organs are the result of *in vivo* proteolysis. It is likely that a trypsin-like enzyme cleaves the $\sim 85K$ and $\sim 75K$ structural proteins into highly antigenic proteins with MW's between 20-30K, similar to those seen with virus purified from mink organs. These findings will be pursued during the next year by comparative peptide mapping of *in vivo* and *in vitro* preparations of ADV.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AI 00263-02 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of the ADV Genome

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

M. E. Bloom

Medical Officer

LPVD NIAID

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Persistent Viral Diseases, Hamilton, MT 59840

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:
1.0 0.6 0.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is the study of genome structure and function of the Aleutian disease virus (ADV), a nondefective parvovirus of mink. Further studies on this project have included the molecular cloning in pUC8 of a 1.6 kbp segment (0.56-0.86 map units) of a Danish isolate of ADV (DK ADV). This clone, designated pBM2, corresponds to pBM1, the equivalent segment of ADV-G also cloned in pUC8. Comparisons of the DNA's of these two plasmids have been made: 1) heteroduplex mapping by electron microscopy showed the ADV-G and DK ADV segments were the same size and contained no extended areas of heterology, 2) however, physical mapping with restriction enzymes has shown distinct differences. These recombinant plasmids have also been tested for protein expression in *E. coli* and we found that both pBM1 and pBM2 induce ADV specific products in JM103. Both plasmids induced 55k, 34k, and 27k polypeptides demonstrable by immune "Western" blotting as well as by immunoprecipitation. The interrelationship of the three proteins is under study but preliminary evidence suggested that at least the 27k protein reacts with monoclonal antibodies having specificity for ADV structural proteins. Finally, we have found that ADV DNA from virions purified from mink organs was of unit length and reacted with our ADV recombinant plasmids. Thus, we now have the reagents to begin molecular cloning of DNA from field strains of ADV.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00380-01 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunological Studies on Aleutian Disease of Mink

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

M. E. Bloom

Medical Officer

LPVD NIAID

COOPERATING UNITS (if any)

None

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1.2	1.0	0.2

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 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to perform basic immunological studies in mink infected with the Aleutian disease virus ADV, a nondefective parvovirus. In the past year, a sensitive solid phase radioimmunoassay for ADV antigen and anti-ADV antibodies was developed. The assay could detect approximately 3.2 ng of ADV protein. After experimental infection, antigen could be detected in gut and kidney at days 3-6 and by day 10 antigen was localized in spleen, liver, kidney, lymph nodes, as well as peritoneal exudates and bone marrow cells. Quantitation was confounded by antibody being present in samples after day 6. In addition, using inhibition of binding, a sensitive assay for anti-ADV antibody was developed capable of detecting as little as 5 ng of antibody. Antibody could be detected as early as 3 days after infection and titers up to 1/10⁶ were found in infected mink. Utilizing this RIA for antibody, several important studies were performed: 1) The amount of anti-ADV antibody in hypergammaglobulinemic sera was calculated and found to be from 13-57% of the total gammaglobulin fraction, indicating a polyclonal stimulation of Ig synthesis in infected mink, 2) The affinity of antiviral antibodies from a number of pooled and individual sera was determined to be indicative of high quality antibody, suggesting that the virus-antiviral antibody complexes in AD are caused by high, not low, affinity antibody and, 3) Estimates of the heterogeneity of antiviral antibodies in these same sera suggested that some, but not all, infected mink develop antibodies of restricted heterogeneity; in fact some were as homogeneous as monoclonal antibodies.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00086-06 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Resistance to Graft Versus Host Disease

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

J. L. Portis

Medical Officer

LPVD NIAID

COOPERATING UNITS (if any)

Dr. T. G. Wegman and P. Gamble, University of Alberta, Edmonton, Alberta,
Canada

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1.0	0.5	0.5

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| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Acute and chronic graft versus host reaction (GVHR) are the primary cause of morbidity and mortality after allogeneic and semi-syngeneic bone marrow transplantation in humans. We are studying the genes which influence both of these syndromes in a mouse model. Acute GVHR is seen in unirradiated (B6 X D2)F₁ recipients of $\geq 60 \times 10^6$ parental spleen cells from B6 but not D2 mice. Using standard genetic approaches, we have identified a single dominant non-H-2 gene from B6 mice which promotes acute bone marrow suppression in the recipient. We are currently attempting to identify genetic linkage using recombinant inbred (RI) mice. This gene is fully expressed by B6 but only partially expressed by B10 donor mice and behaves as a dominant gene in (B6 X B10)F₁ donors. Autoimmune phenomena are observed during chronic GVHR induced in (B6 X D2)F₁ recipients of D2 parental cells. We have identified a single recessive non-H-2 gene in D2 mice which promotes the production of auto-antibody by the recipient with specificity for endogenous xenotropic envelope antigens. Progeny testing of (B6 X D2)F₁ X D2 mice has confirmed this hypothesis and linkage studies will soon be carried out in RI mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00264-02 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Structure and Function
of Endogenous Retroviruses Expressed During Differentiation

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

J. L. Portis

Medical Officer

LPVD NIAID

COOPERATING UNITS (if any) Dr. W. J. Britt, Univ. Alabama, School of Medicine, Birmingham, AL; Dr. C. Melfi and Dr. M. Zijlstra, Central Lab of the Netherlands Red Cross Blood Transfusion Service, The Netherlands; Dr. J. A. Levy, Cancer Res. Inst., Univ. California School of Medicine, San Francisco, CA.

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1.0 0.5 0.5

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 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A panel of monoclonal antibodies has identified four serogroups of xenotropic murine retroviruses. We have used these antibodies to serologically and structurally characterize the viruses expressed by lymphohematopoietic tissues. All xenotropic viruses recovered from spleen, bone marrow and embryonic liver belong to group 1. The predominant cell membrane gp70 expressed by spleen and bone marrow cells also belongs to group 1 whereas that expressed by a large population (>60%) of early embryonic liver cells resembles serologically and structurally group 4 viruses. It is this gp70 which is expressed by erythroid cells during an intermediate stage of normal differentiation. This antigen has also been identified on some but not all erythroid cell lines. However, several attempts to recover infectious group 4 virus from these cell populations (including the erythroid cell lines) have been unsuccessful. This suggests that unlike group 1, group 4 gp70 is not encoded by a replication competent provirus. We now have preliminary evidence in a serologic survey of many different hematopoietic cell lines that myeloid specific retroviral products may exist. The use of these antibodies specific for endogenous viruses is revealing a serologic heterogeneity not previously appreciated and may provide a tool for identifying genetic loci which are functional in normal cellular differentiation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00199-04 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology of Aleutian Disease

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

R. E. Race

Veterinary Officer

LPVD NIAID

COOPERATING UNITS (if any)

None

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 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to define immune mechanisms and viral characteristics important in the pathogenesis of Aleutian disease (AD). Monoclonal antibodies were used to study antigenic differences between the ADV-G and Utah I ADV strains of ADV. Antibodies with reactivity for tissue derived Utah I ADV or tissue culture derived ADV-G were clearly delineated and new information was obtained regarding the specific antigenic determinants recognized by several mAbs whose precise reactivities were obscure earlier. Antibodies recognizing viral antigens purified from ADV infected cell cultures (ADV-G) recognize the major viral structural proteins of ADV while the viral associated antigen recognized by monoclonal antibodies reacting with tissue extracted ADV (Utah I) are low molecular weight proteins probably resulting from *in vivo* proteolysis of larger precursors. The affinities of the mAbs for virus strains were determined as well as the relative epitope densities recognized by individual mAbs. Recently developed mAb's against a Danish ADV strain, a Canadian ADV strain and two additional American strains are yet to be fully characterized but may provide specific markers for individual isolates and allow more precise information regarding the differing capacities of the individual strains to cause overt disease in mink.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00265-02 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology of Scrapie Virus Infection

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

R. E. Race

Veterinary Officer

LPVD NIAID

COOPERATING UNITS (if any)

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 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The immediate goal of this project continues to be the establishment of high titered scrapie infected tissue culture cell lines. Scrapie is a naturally occurring spongiform encephalopathy of sheep and goats which causes clinical and pathological changes similar to those of Creutzfeldt-Jakob and Kuru diseases of man. Scrapie grows to high titer in mouse lymphoid tissue and brain but has never been passaged at high titer in any in vitro system. Adaptation of the agent to cell culture will permit detailed characterization of the agent(s) and provide a system for better definition of the pathogenesis of these diseases. We have utilized two approaches designed to provide infected in vitro cell lines. In one approach, scrapie infected mice are injected with one of several splenotropic tumor cell lines. Once tumors are established in vivo, they are explanted, maintained in vitro and assayed for the presence of scrapie agent. The second approach utilizes hybridoma technology. Spleen cells from scrapie infected mice are fused with a myeloma cell line. If a scrapie infected cell was a partner in the fusion, persistently infected lines might result. Preliminary results suggest that the hybridoma approach was unsuccessful and results of the tumor experiments are not yet available. Other experiments designed to identify scrapie target cells in mouse spleen indicate that only one in one thousand or more cells is infected. It is, therefore, likely that if a particular cell is preferentially infected, it is a subpopulation of one of the major phenotypes (T or B) now known and that currently available cell separation techniques are not likely to isolate them in sufficiently pure form to allow their characterization. Attempts to adapt the agent to a tumor system will therefore be emphasized.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00266-02 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Structure of Murine Retroviruses

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

L. H. Evans

Senior Staff Fellow

LPVD NIAID

COOPERATING UNITS (if any)

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TOTAL MANYEARS:

1.0

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0.5

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0.5

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- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The current emphasis of this project is the identification and characterization of endogenous sequences which recombine with ecotropic murine leukemia viruses (MuLV's). The resulting recombinant viruses, termed mink cell focus-forming viruses (MCF's) are thought to be involved in leukemia induction by MuLV's. The initial studies indicated that an erythroleukemia virus and a lymphatic leukemia virus selectively recombined with different sets of endogenous proviral sequences to give rise to MCF's in NFS mice. Further studies suggest that endogenous ecotropic viruses recombine with sequences distinct from either the erythroleukemia or lymphatic leukemia viruses. The oncogenicity of the MCF's derived from erythroleukemia and lymphatic leukemia virus was examined. MCF's derived from lymphatic leukemia viruses were found to be oncogenic in NFS and AKR mice whereas MCF's derived from the erythroleukemia virus did not induce disease in these strains. Detailed structural analyses of the viral genomes indicate that the MCF's derived from lymphatic leukemia viruses inevitably derive their p15(E) genes and terminal sequences from the ecotropic parent whereas recombinants with the erythroleukemia virus frequently derive their p15(E) gene from endogenous mouse sequences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00267-02 LPVD

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Endogenous Murine Retroviruses and Leukemogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

M. W. Cloyd Senior Staff Fellow LPVD NIAID

COOPERATING UNITS (if any) Dr. J. Hartley, National Institutes of Health, Bethesda, MD; Dr. Sisir Chattopadhyay, National Institutes of Health, Bethesda, MD; Dr. Paul Hoffman, Johns Hopkins Univ., Baltimore, MD; Dr. W. Hazeltine and J. Lenz, Harvard Univ., Cambridge, MA

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1.0	0.5	0.5

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| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input checked="" type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this program is the understanding of leukemogenesis in mice. Two interrelated areas are under investigation: 1) Determination of the elements (including viruses) and processes involved in development of murine lymphoma or leukemia, and 2) Characterization of the types, properties, and functions of endogenous retroviruses in mice and the mechanisms of their regulation, expression, and in the case of the recombinant (MCF) viruses, their generation. These projects, utilizing biological, genetic, serological, and molecular approaches, have helped to unravel some of the details of lymphomagenesis in AKR mice and have defined many of the biological properties of MCF viruses. The studies this year have focused on 1) Further biological characterization of MCF viruses in the determination of their host range, 2) Detailed genetic analysis of host regulation of AKR MCF induction of lymphoma, 3) Cell-virus interactions *in vivo* using lymphomagenic and nonlymphomagenic AKR-type ecotropic viruses and intragenomic recombinants constructed between them, 4) Examination of the genetic sequences of the mouse which recombine with different ecotropic viruses to generate MCF viruses, and 5) Differential viral gene expression of leukemia cells as reflected by env antigen presentation. Major findings from this work include: 1) The host range of MCF viruses was found to be distinct from that of other classes of murine retroviruses, which supported the recent contention that MCF's are a distinct virus class, 2) Gene conveying resistance to MCF induction of lymphoma was conclusively demonstrated in crosses of AKR with NFS, 3) The promotor region of the genome of lymphomagenic AKV-type virus was shown to be the important element for cellular specificity, replication, and oncogenicity, 4) Ecotropic viruses recombine in a specific manner with different proviral sequences of the mouse to generate MCF viruses, and 5) MCF viral env gene products and not xenotropic were expressed on spontaneous or virus-induced leukemias and the type of MCF protein was determined by the mouse strain or ecotropic virus used.

EPIDEMIOLOGY BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
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Annual Report
Epidemiology Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1982, to September 30, 1983

RESEARCH HIGHLIGHTS

The June 18, 1982, Science Report on our discovery of the Lyme disease agent and on the application of the indirect immunofluorescence test as a diagnostic tool for Lyme disease and related disorders became the subjects of numerous press articles that hailed our findings as a significant and exciting breakthrough in medical research. This was echoed in this country as well as in Europe by research scientists and clinicians (dermatologists, rheumatologists) who were eager to either obtain subcultures of the causative agent for their own research and diagnostic purposes or to offer for serologic testing sera of patients with suspected Lyme disease.

As anticipated, our findings also rekindled interest for epidemiological and ecological investigations.

The following sections summarize this year's developments in our research program. Some of the findings were reported in 18 publications and in an additional 16 papers that are currently in press.

RICKETTSIOSSES

As in previous years, we functioned as a World Health Organization Center for Rickettsial Reference and Research and provided reference reagents, guidance and training to investigators and staff of various domestic and foreign health agencies.

Comparative immunochemical analyses (SDS-PAGE, western blot) between the "Swiss agent", a spotted fever group rickettsia isolated from up to 12% of Ixodes ricinus from Switzerland, and the three in Europe prevalent spotted fever group rickettsiae, i.e. R. conorii, R. slovaca, and R. sibirica, indicated that the "Swiss agent" differs in antigenic makeup. The name, Rickettsia helvetica, is being proposed (PETER, BURGDORFER, WILLIAMS).

Dogs exposed to western strains of R. rickettsii were shown to develop a mild disease with immune responses characterized by low titers of antibodies. In contrast, dogs infected with eastern strains of R. rickettsii developed severe clinical manifestations compatible to those observed in man, and, following recovery, usually exhibited enormously high titers of antibodies. These differences, not known to occur in man, appear to be related to different pathogen-vector-host relationships of western and eastern strains of the spotted fever agent (BURGDORFER, GREENE, PEACOCK).

Rickettsioses (Cont'd)

Virulent R. rickettsii isolated either from patients or from ticks in western Montana, were shown to have an adverse affect on the biological development of the tick vector, Dermacentor andersoni. These affects appear to be related to the massive rickettsial development that is accelerated by elevated body temperatures (fever) of an infected host. This phenomenon, undoubtedly, represents an important factor responsible for the low rates of R. rickettsii-infected ticks in nature (BURGDORFER, PETER).

An outbreak (4 patients) of boutonneuse fever near Geneva, Switzerland, was related to bites of Rhipicephalus sanguineus ticks that had been imported on a pet dog from southern France or Italy where boutonneuse fever commonly occurs. A survey of the household maintaining the dog revealed heavy tick infestations in every room. Hemolymph testing of ticks resulted in 30 (40%) of 75 nymphal and adult ticks with a rickettsia indistinguishable from R. conorii the causative agent of boutonneuse fever (PETER, BURGDORFER, AESCHLIMANN).

Ticks of the Ixodes ricinus complex, namely I. dammini and I. pacificus, have been found to harbor in their intestinal and genital tissues a rickettsialike symbiote that like the East side agent in Dermacentor andersoni interferes with the development of the spotted fever agent, R. rickettsii (BURGDORFER, TODD).

Q FEVER

An evaluation of the serological profiles of the three well-characterized clinical entities of Q fever in humans, namely Primary disease, granulomatous hepatitis, and endocarditis, showed that persistently elevated IgG and IgM antibodies to phase II antigens were characteristics of granulomatous hepatic disease, whereas increased IgG antibody titers to both phase II and phase I antigens characterized endocarditis. More important, the presence of specific IgA anti-phase II-I was diagnostic for Q fever endocarditis. Specific diagnostic titers of IgA antibodies were not found in sera from patients with primary or hepatic disease. IgM rheumatoid factor (RF) was present in sera from patients with chronic Q fever but not in sera from patients with primary Q fever infections. The results of the study indicate that (1) the choice of antibody assay method is critical for proper evaluation of specific antibody levels to C. burnetii, (2) at least three clinical entities of Q fever can be distinguished by IFA of specific IgG, IgM, and IgA; and (3) autoimmune mechanisms are induced during primary and chronic Q fever. The presence of specific humoral C. burnetii IgA antibody in patients with Q fever endocarditis is diagnostic (PEACOCK, WILLIAMS, THOMAS).

LYME DISEASE

Spirochetes indistinguishable from those recovered from Ixodes dammini from Shelter Island (NY) were isolated from the bloods, skin lesion (ECM) or cerebrospinal fluid of 5 patients with symptoms of Lyme disease thus confirming the previously postulated spirochetal etiology of this disease (STEERE, BENACH, BURGDORFER, BARBOUR).

Lyme Disease (Cont'd)

Meningo-radiculitis, lymphadenitis, and acrodermatitis chronicum atrophicans are clinical manifestations occurring following bites of I. ricinus in Europe. The etiology of these diseases is as yet unknown. Indirect immunofluorescence of sera from such patients suggests that these disorders are expressions of the I. ricinus-associated spirochete (BURGDORFER, WEBER).

Ecological studies on Shelter Island and in Connecticut resulted in isolation of spirochetes from the bloods of one white-tailed deer (Odocoileus virginianus), one raccoon (Procyon lotor), and 6 white-tailed mice (Peromyscus leucopus) - mammals that serve as hosts of I. dammini (MAGNARELLI, ANDERSON, BOSLER, BURGDORFER, BARBOUR).

In Oregon and California where cases of Lyme disease had occurred, the Pacific Coast tick, I. pacificus, was proven to be a vector of spirochetes indistinguishable from those associated with I. dammini in the eastern U.S. (BURGDORFER, LANE, GRESBRINK).

A bacteriophage with a B-3 morphology was detected by electron microscopy in spirochetes isolated from I. dammini ticks. It has a 40 to 50 nm elongated head and a tail 50 to 70 nm in length. It appears devoid of collars or kite-tail structure. This is the first bacteriophage associated with a tick-borne spirochete pathogenic to man (HAYES, BURGDORFER, BARBOUR).

The ELIZA test was found to be a useful tool for detecting antibodies to Lyme disease spirochetes. Suspensions of whole organisms or sonicated organisms proved stable as antigens for at least 6 months when stored at 4° C. Sera of patients having had syphilis cross reacted against the Lyme disease spirochetes in titers of 1:20 and higher (using an antigen dilution of 1:40, 960) (THOMAS, BARBOUR, BURGDORFER).

GENESIS OF CHRONIC DISEASE

As reported recently (J. Inf. Dis. 146:657, 1982), observations on the temporal distribution of scrapie virus in naturally infected Suffolk sheep outline the main events in the infection and offer clues about the mode of transmission. The early appearance of virus in tonsil, retropharyngeal and mesenteric lymph nodes, and intestine suggest that primary infection occurs along the alimentary tract, either prenatally from virus in amniotic fluid (vertical transmission) or postnatally from virus in a contaminated environment. No evidence of subclinical infection was found in clinically normal high-risk sheep beyond the age when scrapie is most prevalent (HADLOW, KENNEDY, RACE).

SYSTEMATIC OF TICKS

Two new species of ticks were described, and Ornithodoros muesebecki, the vector of zirqa virus that severely debilitates oil field workers on islands in the Arabian Gulf, was redescribed (CLIFFORD, KEIRANS).

Annual Report
Epidemiology Branch
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National Institute of Allergy and Infectious Diseases
October 1, 1982, to September 30, 1983

ADMINISTRATIVE REPORT

Currently, the Epidemiology Branch is manned by 4 Ph.D. level scientists, all tenured, including 2 in the Arthropod-Borne Diseases Section and one each in the Epidemiology and Histopathology Sections. In addition, there are 4 supporting staff. Since May 1, 1982, Dr. W. Burgdorfer has been Acting Chief of the EB.

Anticipated transfer of RML's tick collection and assignment of the two curators, Drs. C. M. Clifford and J. E. Keirans, to the Smithsonian Institution at Silver Hill, MD, became effective August 1, 1983. Such a transfer not being in his best interest, Dr. C. M. Clifford submitted a request for retirement as of October 1, 1983. To assist both curators in packing the huge collection of ticks, related files, correspondence, and literature, Ms. Dorothy Palmer was hired on a temporary basis.

During the year, Mr. R. E. Overton and Mr. A. J. Mavros, Biological Laboratory Technicians retired after 23 and 37 years of government service, respectively.

Dr. Olivier Peter, University of Neuchatel, Switzerland, was granted a second year NIH Visiting Fellowship, effective January 4, 1983, to receive training in molecular and immunochemical investigations of rickettsiae.

Guest workers in Dr. Burgdorfer's unit included Drs. B. R. Norment, Department of Entomology, Mississippi State University, Mississippi State, May 13-27, 1983, A. Farhang-Azad, Department of Microbiology, University of Maryland, Baltimore, MD, June 9-18, 1983, and P. E. Lavoie, M.D., San Francisco, Ca. Dr. L. Logan, USDA, Northeastern Region Plum Island Animal Disease Center, New York will be a guest worker in Dr. Burgdorfer's laboratory September 11-13, 1983.

Annual Report
Epidemiology Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1982, to September 30, 1983

HONORS AND AWARDS

The following activities reflect recognition of EB by peers and the scientific community:

Editorial Boards of Journals

Dr. W. Burgdorfer - Acta Tropica: Current Topics in Pathogen-Vector-Host Research

Dr. W. J. Hadlow - Fundamental and Applied Toxicology

Drs. Burgdorfer, Clifford, Hadlow and Keirans reviewed manuscripts for the following journals: J. Med. Entomol.; J. Econ. Entomol.; J. Wildlife Dis.; J. Infect. Dis.; Science; Vet. Pathol.; J. Parasitol.; Infect. Immun.; Acta Tropica.

Professional Posts

Dr. W. Burgdorfer continued to serve as Acting Chief, EB, RML; continued to serve as President of the American Society for Rickettsiology and Rickettsial Diseases; continued to serve as Director of the WHO Reference Center for Rickettsiae and Rickettsial Diseases at RML.

Dr. W. J. Hadlow appointed to the Advisory Board of the Charles Louis Davis Foundation for the Advancement of Veterinary Pathology; appointed to the Committee on Diseases of Sheep and Goats of the U.S. Animal Health Association; continued as Adjunct Professor of Veterinary Pathology, Washington State University; continued as member of the Burroughs Wellcome Fellowship Selection Committee of the American College of Veterinary Pathologists.

Invited Lectures and Participation in Meetings and Symposia

Dr. W. Burgdorfer - presented on invitation lecture on "Erythema chronicum migrans - a tickborne spirochosis" at the 162nd Annual Meeting of the Swiss Academy of Sciences, Oct. 7-10, 1982, Basel, Switzerland; invited to deliver the SAMUEL W. JOHNSON Memorial Lecture at the Annual Plant Science Day of the Connecticut Agricultural Experiment Station, New Haven, CT., Aug. 10, 1983; invited to present paper on "Ticks and Lyme Disease" at the 14th Annual Conference of the Society of Vector Ecologists, San Diego, CA., Dec. 14-16, 1983; invited to write chapter on "Borreliae" for the 4th edition of ASM Manual of Clinical Microbiology; invited to write chapter on "Transovarial transmission of arthropod-borne viruses and rickettsiae" for Current Topics in Pathogen-Vector-Host Research.

Honors and Awards (Cont'd)

Dr. W. J Hadlow presented lecture on "Slow Viral Diseases" at Kansas State University College of Veterinary Medicine, Manhattan, KS., March 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI AI 00061-21 EB

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Natural History of Tick-borne Rickettsiae and Their Public Health Significance

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Willy Burgdorfer Res. Entomologist (Med) EB NIAID

COOPERATING UNITS (if any)

Dr. R. A. Elston, Battelle Marine Research Lab. WA:

Dr. C. Greene, Univ. of Georgia, Athens, GA; Dr. B. Klein, Dept. Health and Social Serv., Madison, WI; Dr. A. Aeschlimann, Univ. of Neuchatel, Switzerland.

LAB/BRANCH

Epidemiology Branch, (RML), Hamilton, MT 59840

SECTION

Arthropod-Borne Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

2.5

PROFESSIONAL:

1.2

OTHER:

1.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project concerns studies of Rocky Mountain spotted fever and other tick-borne rickettsial diseases in the United States and in certain other countries with emphasis on the ecology, identification, and characterization of rickettsiae and on their relationship(s) to the respective tick vectors. Source material for experimental comparative studies is obtained through collaboration with outside agencies. The project also considers the cellular and subcellular aspects of interaction between tick-borne rickettsiae and their vectors, particularly the mechanism(s) of interference and the factors responsible for changes in the agents' pathogenicity.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00063-13 EB

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immune Responses to Rickettsial Infections

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

M. G. Peacock, Microbiologist, EB, NIAID

COOPERATING UNITS (if any)

Ruth S. Faulkner, Dept. of Microbiology, Public Health Laboratories,
Halifax, Nova Scotia

LAB/BRANCH

Epidemiology Branch, (RML), Hamilton, MT 59840

SECTION

Arthropod-borne Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to study immune responses in man and experimental animals to natural and experimental rickettsial infections. Serological parameters were compared in 15 cases of Coxiella burnetii infection comprising 5 cases of primary Q fever, chronic granulomatous hepatitis, and endocarditis. The diagnosis was made on the basis of clinical history and serology and on the isolation of C. burnetii phase I organism from biopsy specimens of liver and bone marrow from two patients with granulomatous hepatitis and from the aortic valve vegetations of five patients with endocarditis. The temporal sequences of immunoglobulin levels, rheumatoid factor, and specific antibody responses to phase II and phase I antigens of C. burnetii were evaluated as predictive correlates of the 3 Q fever entities by microagglutination (MA), complement fixation (CF), and indirect immunofluorescence tests (IFA). Serum levels of immunoglobulin classes G, M, and A were variable in all entities of Q fever. Increased mean levels of immunoglobulin G (IgG) and IgA were noted with chronic disease in the sera of some patients, whereas IgM levels were not significantly elevated in chronic disease but not in primary Q fever. The high phase-specific IgA antibody titers in the indirect microimmuno-fluorescence test were diagnostic for endocarditis. This project also evaluates a recently developed serological technique (ELISA) for the diagnosis of bacterial (especially rickettsial) diseases. It also provides serological support to other RML units and includes serodiagnosis of bacterial, rickettsial, or viral diseases under investigation, the monitoring of employees, visiting investigators, and experimental animals for antibodies against various infectious agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZOI AI 00065-10

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Antigens and Classification of the Rickettsiae

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

R. N. Philip Medical Director EB NIAID

COOPERATING UNITS (if any)

Scott W. Gordon, Ohio Department of Health, Columbus, Ohio

LAB/BRANCH

Epidemiology Branch, Hamilton, MT 59840

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Dr. Philip retired. Project has been transferred to R. L. Anacker (See LMSF annual report).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AI 00069-22 EB

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Systematics and Vector Relationships of Certain Parasitic Arthropods

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

C. M. Clifford Scientific Director EB NIAID

COOPERATING UNITS (if any)

Drs. H. Hoogstraal, NAMRU-3; D. Sonenshine and P. Homsher, Old Dominion Univ.; C. Centurier, Univ. of Munich; Miss Jane Walker, Div. of Vet. Services, Onderstepoort; Mr. Rupert Pegram, Tick Diseases Unit, Lusaka, Zambia.

LAB/BRANCH

Epidemiology Branch (RML), Hamilton, MT 59840

SECTION

Arthropod-Borne Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

2.6

PROFESSIONAL:

2.0

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The activities of this project currently comprise four main functions:

- 1) Identification of ticks received from various individuals and government agencies throughout the world. Only one other institution in the world is capable of performing this service.
- 2) Systematic study of certain groups of parasitic arthropods. The foremost tool in systematic studies, the scanning electron microscope, has greatly aided in elucidating taxonomic concepts in acarines actually or potentially involved in transmission of disease agents.
- 3) Retrieval and use of tick data in the Smithsonian data retrieval system. Installation of a mini-computer-word processor system at RML to communicate with NIAID and Smithsonian computers.
- 4) Colonization of medically important arthropod vectors, which are furnished to outside investigators.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI AI 00081-22 EB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Epidemiology of Human Infection of Special Interest

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

R. N. Philip Medical Director EB NIAID

COOPERATING UNITS (if any)

Dr. Catharine Wilfert, Duke University School of Med., Durham, N.C.

LAB/BRANCH

Epidemiology Branch, Hamilton, MT 59840

SECTION

Epidemiology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00082-22 EB

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Relation of Viruses to the Genesis of Chronic Disease

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

W. J. Hadlow, Research Veterinarian (Path.), EB/NIAID

COOPERATING UNITS (if any)

Dr. S. B. Prusiner, Department of Neurology, University of California, San Francisco.

LAB/BRANCH

Epidemiology Branch (RML), Hamilton, MT 59840

SECTION

Histopathology Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two naturally occurring viral infections of domestic animals are used as models for studying the unusual host-virus interactions that result in slowly evolving diseases. These are (1) scrapie of sheep and goats and (2) Aleutian disease of ranch mink. Scrapie is a degenerative disease of the brain (polioencephalopathy) caused by an unconventional virus (scrapie agent) replicating in central nervous tissue. Aleutian disease, caused by a nondefective parvovirus, is mainly a chronic renal disease mediated by infectious immune complexes that become deposited in the glomeruli. Simple methods of clinical observation, serology, virology, animal inoculation, and anatomic pathology are used to obtain information on the pathogenesis and natural history of each disease. Such information helps characterize each disease as a nosologic entity and provides a better understanding of the way it comes about. In addition, such information on the animal diseases will help determine the relation of slow viral infection to the genesis of chronic disease in man. Included in the project is one such disease, Creutzfeldt-Jakob disease, for which scrapie is the prototype.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI AI 00084-14 EB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vector-Pathogen Relationships

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

C. E. Yunker Scientist Director EB NIAID

COOPERATING UNITS (if any) Dr. K. Abdel-Wahab, Al Azhar Univ., Cairo; Dr. M. Y.

Kamel, National Research Center, Cairo; Dr. A. Main, Yale Univ. School of Med.,
New Haven, CT; Dr. C. Chastel, Faculte de Medecine, Brest; Dr. M. Takahashi,
Nat. Inst. Health, Tokyo; Dr. C. Centurier, Univ. of Munich.

LAB/BRANCH

Epidemiology Branch, Hamilton, MT 59840

SECTION

Arthropod-Borne Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZOI AI 00200-04-EB

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunobiology of Relapsing Fever

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

H. G. Stoerner Asst. Sci. Director, NIAID (RML) (Retired 1981)

COOPERATING UNITS (if any)

None

LAB/BRANCH

Office of the Scientific Director, NIAID

SECTION

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: PROFESSIONAL: OTHER:

CHECK APPROPRIATE BOX(ES)

- | | | |
|---|--|--------------------------------------|
| <input type="checkbox"/> (a) Human subjects | <input type="checkbox"/> (b) Human tissues | <input type="checkbox"/> (c) Neither |
| <input type="checkbox"/> (a1) Minors | | |
| <input type="checkbox"/> (a2) Interviews | | |

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZOI AI 00268-02 EB

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ecology of Lyme Disease and Related Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and Institute affiliation)

W. Burgdorfer Res. Entomologist (Med) EB NIAID

COOPERATING UNITS (if any).

Dr. J. Benach, NY State Health Dept., Dr. A. C.
 Steere, Yale Univ., New Haven, CT., Dr. R. S. Lane, Univ. CA., Berkeley, CA.,
 Dr. J. P. Davis, Dept. Health & Social Sciences, Madison, WI.; Dr. A.
Aeschlimann, Univ. Neuchatel, Switzerland.

LAB/BRANCH

Epidemiology Branch, (RML), Hamilton, MT 59840

SECTION

Arthropod-Borne Diseases Section

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS: PROFESSIONAL:

1.5

0.8

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to determine the natural history of the recently discovered and isolated causative agent of Lyme disease and related disorders. The relationship(s) between the spirochete and its various Ixodes spp. tick vectors (I. dammini, I. pacificus, I. scapularis, I. ricinus) is being determined by establishing through conventional as well as transmission and scanning electron microscopy a) the development of the spirochete within the ticks, and b) the mode(s) of transmission to vertebrate hosts. In cooperation with outside agencies, tick/spirochete surveys are being conducted to determine prevalence of infected ticks in endemic foci. Similarly, the natural source(s) for infection of ticks is being evaluated first serologically (indirect immunofluorescence) and subsequently through recovery of the spirochete from serologically implicated hosts.

ROCKY MOUNTAIN OPERATIONS BRANCH
Rocky Mountain Laboratories
Hamilton, Montana
1983 Annual Report
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<u>Project Number</u>	
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Annual Report
Operations Branch
Rocky Mountain Laboratories
Hamilton, Montana
National Institute of Allergy and Infectious Diseases
October 1, 1982 to September 30, 1983

Introduction

The Branch provides all services necessary to the professional staff in the pursuit of their investigations. This support covers the following areas: procurement, personnel, communications, library services, secretary backup service, grounds care, custodial, security, media preparations, waste disposal, glassware refinishing, photography, animal rearing and care, motor pool, operation of power plants, and full maintenance in every area except electronics.

General Overview of the Responsibilities of Operations Branch

The fiscal and procurement department manages a budget of \$1,180,000. Payroll is not included in this figure. It covers only the purchase of supplies and minor equipment used in the operation of the laboratories. Timekeeping and the correction of errors in the payroll are also handled in the unit.

Personnel handles all actions and advises on personnel matters. This department also is charged with the operation of the Comprehensive Employment Training Act (CETA) in association with the local Montana State Employment Office. Through the year, we have averaged one person on this program, serving a two-month appointment. The maximum time a person may spend on the program is two months. Hence, we are constantly interviewing and employing people under the program. Also handled by Personnel are persons under the following programs: Stay-in-School, Work Study, Government Summer Program, Visiting Program, and students studying for advanced degrees.

Custodial services are provided in the five laboratory buildings daily. Security is provided in the form of a guard on duty every night of the year.

Most of the media used in the research laboratories is prepared in a special laboratory by a technician. All glassware is cleaned and sterilized in the glassware department for reuse in the laboratories.

The Graphic Arts Department provides full professional services necessary in the laboratories with the exception of medical artistry.

The Animal Unit raises 12 strains of mice, 8 strains of hamsters, and 1 colony of microtus. They breed and raise approximately 90,000 animals a year. An additional 7,000 animals are purchased annually from outside sources, including mink, sheep, rabbits, mice, and hamsters. After rearing, care is provided for these animals while they are under experiment.

The maintenance department, through the power plant, provides heat, steam, air and vacuum to the laboratories. Also provided are air conditioning, compressed air, and demineralized and distilled water. A motor pool consisting of 10 vehicles is maintained. Grounds care, including snow removal, is provided.

With the exception of the electronics work, all maintenance is done by the staff. This includes plumbing, electrical, sheet metal, carpentry, and refrigeration, including ultra low temperature boxes.

Labor management work is handled by the Chief of the Branch.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AI 00201-04 RMOB

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Characterization of Integrated Viral Genomes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Claude F. Garon, Section Head, RMOB/NIAID

COOPERATING UNITS (if any)

Malcolm A. Martin, LMM/NIAID; Takis S. Papas, LMO/NCI; Charles J. Sherr,
St Jude's Hospital, Memphis; R. Silver, FDA.

LAB/BRANCH

Rocky Mountain Operations Branch (RML), Hamilton, MT 59840

SECTION

Electron Microscopy

INSTITUTE AND LOCATION

NIAID, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

1.5

PROFESSIONAL:

0.8

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several classes of viruses form stable associations with their hosts by the integration of one or more copies of the viral genome into host cell DNA. Retroviruses provide a unique and important system for the study of integrative recombination since exogenously acquired genomes are integrated with high efficiency at a specific site in the viral genome, but at a large number of sites in the host chromosome. Molecular clones of several newly integrated retroviral genomes were produced in either plasmid or bacteriophage cloning vehicles using approved recombinant DNA techniques and were characterized using electron microscope heteroduplex and R-looping methods. These studies have not only provided information on the structural arrangement of both integrated viral and flanking cellular sequences but also have confirmed the presence of transformation specific sequences in normal, uninfected host cells. The major objective of these studies has been the application of physical and biochemical techniques to assess the influence of flanking cellular sequences on subsequent viral function and to define in molecular terms those events which take place during integrative recombination.

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Bethesda, Md. 20205



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